PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/24, C12P 21/02, C07K 13/00, C12N 1/21, A61K 37/02, C12N 15/62

A2

(11) International Publication Number: WO 94/12639

(43) International Publication Date: 9 June 1994 (09.06.94)

(21) International Application Number: PCT/US93/11198

(22) International Filing Date: 22 November 1993 (22.11.93)

(30) Priority Data:
07/981,044 24 November 1992 (24.11.92) US

(60) Parent Application or Grant
(63) Related by Continuation
US 07/981,044 (CIP)
Filed on 24 November 1992 (24.11.92)

(71) Applicants *(for all designated States except US)*: G. D. SEARLE & CO. [US/US]; Corporate Patent Department, P.O. Box 5110, Chicago, IL 60680-5110 (US). THE MONSANTO COMPANY [US/US]; 800 North Lindbergh Boulevard, St.Louis, MO 63166 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BAUER, S., Christopher [US/US]; Box 108A, RR #1, New Haven, MO 63068 (US). ABRAMS, Mark, Allen [US/US]; 7723 Blackberry Avenue, St. Louis, MO 63130 (US). BRAFORD-GOLDBERG, Sarah, Ruth [US/US]; 4111 West Pine #10, St. Louis, MO 63108 (US). CAPARON, Maire, Helena [IE/US]; 109 Beechwood Court, Chesterfield, MO 63017 (US). EASTON, Alan, Michael [US/US]; 2317 Seven Pines Drive #7, Maryland Heights, MO 63146 (US). KLEIN, Barbara, Kure [US/US]; 12917 Topping Estates, Town and Country, MO 63131 (US). MCKEARN, John, Patrick [US/US]; 1430 Highway C., Glencoe, MO 63038 (US). OLINS, Peter, O. [GB/US]; 17507 Summit View, Glencoe, MO 63038 (US). PAIK, Kumman [US/US]; 1021 Alpine Ridge, Ballwin, MO 63021 (US). POLAZZI, Joseph, O. [US/US]; 15570 Century Lake Drive, Chesterfield, MO 63017 (US). THOMAS, John, Warren [US/US]; 13426 Mason Valley Court, Town and Country, MO 63131 (US).

(74) Agents: BENNETT, Dennis, A. et al.; G. D. Searle & Co., Corporate Patent Department, P.O. Box 5110, Chicago, IL 60680-5110 (US).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: INTERLEUKIN-3 (IL-3) MUTANT POLYPEPTIDES

(57) Abstract

The present invention relates to a recombinant human interleukin-3 (hIL-3) variant or mutant proteins (muteins). These hIL-3 muteins contain one or more amino acid substitutions and may also have amino acid deletions at both the N- and C-termini. The invention also relates to pharmaceutical compositions containing the hIL-3 muteins and methods for using them. Additionally, the present invention relates to recombinant expression vectors comprising nucleotide sequences encoding the hIL-3 muteins, related microbial expression systems, and processes for making the hIL-3 muteins using the microbial expression systems. Included in the present invention are deletion mutants of hIL-3 in which from 1 to 14 amino acids have been deleted from the N-terminus, and from 1 to 15 amino acids C corresponding to residues 119 to 133) have been deleted from the C-terminus, and which also contain one to three amino acid substitutions in the polypeptide. These hIL-3 mutant polypeptides may have biological activities similar to or better than hIL-3 and, in some cases, may also have an improved side effect profile.

```
    1                   5                  10
ATG GCT CCA ATG ACT CAG ACT ACT TCT CTT AAG ACT TCT
Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser

        15                  20                  25
TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA
Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr

                30                  35
CAC TTA AAG CAG CCA CCT TTG CCT TTG CTG GAC TTC AAC
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn

    40                  45                  50
AAC CTC AAT GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn

            55                  60
AAC CTT CGA AGG CCA AAC CTG GAG GCA TTC AAC AGG GCT
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala

65                  70                  75
GTC AAG AGT TTA CAG AAT GCA TCA GCA ATT GAG AGC ATT
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile

        80                  85                  90
CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG GCC
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala

                95                  100
GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp

    105                 110                 115
TGG AAT GAA TTC CGT CGT AAA CTG ACC TTC TAT CTG AAA
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys

            120                 125
ACC TTG GAG AAC GCG CAG GCT CAA CAG ACC ACT CTG TCG
Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser

130
CTA GCG ATC TTT TAA TAA  [SEQ ID NO:144]
Leu Ala Ile Phe END END  [SEQ ID NO:128]
```

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

INTERLEUKIN-3 (IL-3) MUTANT POLYPEPTIDES

This is a continuation-in-part of United States Application Serial No. 07/981,044 filed November 24, 1992 which is incorporated herein by reference.

Field of the Invention

The present invention relates to mutants or variants of human interleukin-3 (hIL-3) which contain one or more amino acid substitutions and which may have portions of the native hIL-3 molecule deleted. These hIL-3 single and multiple mutation polypeptides retain one or more activities of native hIL-3 and may also show improved hematopoietic cell-stimulating activity and/or an improved activity profile which may include reduction of undesirable biological activities associated with native hIL-3.

Background of the Invention

Colony stimulating factors (CSFs) which stimulate the differentiation and/or proliferation of bone marrow cells have generated much interest because of their therapeutic potential for restoring depressed levels of hematopoietic stem cell-derived cells. CSFs in both human and murine systems have been identified and distinguished according to their activities. For example, granulocyte-CSF (G-CSF) and macrophage-CSF (M-CSF) stimulate the in vitro formation of neutrophilic granulocyte and macrophage colonies, respectively while GM-CSF and interleukin-3 (IL-3) have broader activities and stimulate the formation of both macrophage, neutrophilic and eosinophilic granulocyte colonies. IL-3 also stimulates the formation of mast, megakaryocyte and pure and mixed erythroid colonies.

Because of its ability to stimulate the

proliferation of a number of different cell types and to support the growth and proliferation of progenitor cells, IL-3 has potential for therapeutic use in restoring hematopoietic cells to normal amounts in those cases where the number of cells has been reduced due to diseases or to therapeutic treatments such as radiation and chemotherapy.

Interleukin-3 (IL-3) is a hematopoietic growth factor which has the property of being able to promote the survival, growth and differentiation of hematopoietic cells. Among the biological properties of IL-3 are the ability (a) to support the growth and differentiation of progenitor cells committed to all, or virtually all, blood cell lineages; (b) to interact with early multipotential stem cells; (c) to sustain the growth of pluripotent precursor cells; (d) to stimulate proliferation of chronic myelogenous leukemia (CML) cells; (e) to stimulate proliferation of mast cells, eosinophils and basophils; (f) to stimulate DNA synthesis by human acute myelogenous leukemia (AML) cells; (g) to prime cells for production of leukotrienes and histamines; (h) to induce leukocyte chemotaxis; and (i) to induce cell surface molecules needed for leukocyte adhesion.

Mature human interleukin-3 (hIL-3) consists of 133 amino acids. It has one disulfide bridge and two potential glycosylation sites (Yang, et al., CELL 47:3 (1986)).

Murine IL-3 (mIL-3) was first identified by Ihle, et al., J. IMMUNOL. 126:2184 (1981) as a factor which induced expression of a T cell associated enzyme, 20 - hydroxysteroid dehydrogenase. The factor was purified to homogeneity and shown to regulate the growth and differentiation of numerous subclasses of early hematopoietic and lymphoid progenitor cells.

In 1984, cDNA clones coding for murine IL-3 were isolated (Fung, et al., NATURE 307:233 (1984) and Yokota, et al., PROC. NATL. ACAD. SCI. USA 81:1070 (1984)). The murine DNA sequence coded for a polypeptide of 166 amino acids including a putative signal peptide.

The gibbon IL-3 sequence was obtained using a gibbon cDNA expression library. The gibbon IL-3 sequence was then used as a probe against a human genomic library to obtain a human IL-3 sequence.

Gibbon and human genomic DNA homologues of the murine IL-3 sequence were disclosed by Yang, et al., CELL 47:3 (1986). The human sequence reported by Yang, et al. included a serine residue at position 8 of the mature protein sequence. Following this finding, others reported isolation of Pro^8 hIL-3 cDNAs having proline at position 8 of the protein sequence. Thus it appears that there may be two allelic forms of hIL-3.

Dorssers, et al., GENE 55:115 (1987), found a clone from a human cDNA library which hybridized with mIL-3. This hybridization was the result of the high degree of homology between the 3' noncoding regions of mIL-3 and hIL-3. This cDNA coded for an hIL-3 (Pro^8) sequence.

U.S. 4,877,729 and U.S. 4,959,454 disclose human IL-3 and gibbon IL-3 cDNAs and the protein sequences for which they code. The hIL-3 disclosed has serine rather than proline at position 8 in the protein sequence.

Clark-Lewis, et al., SCIENCE 231:134 (1986) performed a functional analysis of murine IL-3 analogues synthesized with an automated peptide synthesizer. The authors concluded that the stable tertiary structure of the complete molecule was required for full activity. A study on the role of the disulfide bridges showed that

replacement of all four cysteines by alanine gave a molecule with 1/500th the activity as the native molecule. Replacement of two of the four Cys residues by Ala(Cys^{79}, Cys^{140} -> Ala^{79}, Ala^{140}) resulted in an increased activity. The authors concluded that in murine IL-3 a single disulfide bridge is required between cysteines 17 and 80 to get biological activity that approximates physiological levels and that this structure probably stabilizes the tertiary structure of the protein to give a conformation that is optimal for function. (Clark-Lewis, et al., PROC. NATL. ACAD. SCI. USA 85:7897 (1988)).

International Patent Application (PCT) WO 88/00598 discloses gibbon- and human-like IL-3. The hIL-3 contains a Ser^{8} -> Pro^{8} replacement. Suggestions are made to replace Cys by Ser, thereby breaking the disulfide bridge, and to replace one or more amino acids at the glycosylation sites.

EP-A-0275598 (WO 88/04691) illustrates that Ala^{1} can be deleted while retaining biological activity. Some mutant hIL-3 sequences are provided, e.g., two double mutants, Ala^{1} -> Asp^{1}, Trp^{13} -> Arg^{13} (pGB/IL-302) and Ala^{1} -> Asp^{1}, Met^{3} -> Thr^{3} (pGB/IL-304) and one triple mutant Ala^{1} -> Asp^{1}, Leu^{9} -> Pro^{9}, Trp^{13} -> Arg^{13} (pGB/IL-303).

WO 88/05469 describes how deglycosylation mutants can be obtained and suggests mutants of Arg^{54}Arg^{55} and Arg^{108}Arg^{109}Lys^{110} might avoid proteolysis upon expression in Saccharomyces cerevisiae by KEX2 protease. No mutated proteins are disclosed. Glycosylation and the KEX2 protease activity are only important, in this context, upon expression in yeast.

WO 88/06161 mentions various mutants which theoretically may be conformationally and antigenically

neutral. The only actually performed mutations are Met^2 -> Ile^2 and Ile^{131} -> Leu^{131}. It is not disclosed whether the contemplated neutralities were obtained for these two mutations.

WO 91/00350 discloses nonglycosylated hIL-3 analog proteins, for example, hIL-3 ($Pro^8Asp^{15}Asp^{70}$), Met^3 rhul-3 ($Pro^8Asp^{15}Asp^{70}$); Thr^4 rhuL-3 ($Pro^8Asp^{15}Asp^{70}$)and Thr^6 rhuIL-3 ($Pro^8Asp^{15}Asp^{70}$). It is said that these protein compositions do not exhibit certain adverse side effects associated with native hIL-3 such as urticaria resulting from infiltration of mast cells and lymphocytes into the dermis. The disclosed analog hIL-3 proteins may have N termini at Met^3, Thr^4, or Thr^6.

WO 91/12874 discloses cysteine added variants (CAVs) of IL-3 which have at least one Cys residue substituted for a naturally occurring amino acid residue.

SUMMARY OF THE INVENTION

The present invention relates to recombinant human interleukin-3 (hIL-3) variant or mutant proteins (muteins). These hIL-3 muteins contain amino acid substitutions and may also have amino acid deletions at either/or both the N- and C- termini. Preferably, these mutant polypeptides of the present invention contain one to three amino acids which differ from the amino acids found at the corresponding positions in the native hIL-3 polypeptide. The invention also relates to pharmaceutical compositions containing the hIL-3 muteins, DNA coding for the muteins, and methods for using the muteins. Additionally, the present invention relates to recombinant expression vectors comprising nucleotide sequences encoding the hIL-3 muteins, related microbial expression systems, and processes for making the hIL-3 muteins using the microbial expression systems.

The present invention includes mutants of hIL-3 in which from 1 to 14 amino acids have been deleted from the N-terminus and/or from 1 to 15 amino acids have been deleted from the C-terminus, and in which from one to three amino acid substitutions have been made. Preferred muteins of the present invention are those in which amino acids 1 to 14 have been deleted from the N-terminus, or amino acids 126 to 133 have been deleted from the C-terminus, and which both also contain from one to three amino acid substitutions in the polypeptide sequence. These hIL-3 multiple mutation polypeptides may have biological activities similar to or better than hIL-3 and, in some cases, may also have an improved side effect profile, i.e., some muteins may have a better therapeutic index than native hIL-3. The present invention also provides muteins which may function as IL-3 antagonists or as discrete antigenic fragments for the production of antibodies useful in immunoassay and immunotherapy protocols. In addition to the use of the hIL-3 mutant polypeptides of the present invention in vivo, it is envisioned that in vitro uses would include the ability to stimulate bone marrow and blood cell activation and growth before infusion into patients.

Antagonists of hIL-3 would be particularly useful in blocking the growth of certain cancer cells like AML, CML and certain types of B lymphoid cancers. Other conditions where antagonists would be useful include those in which certain blood cells are produced at abnormally high numbers or are being activated by endogenous ligands. Antagonists would effectively compete for ligands, presumably naturally occurring hemopoietins including and not limited to IL-3, GM-CSF and IL-5, which might trigger or augment the growth of cancer cells by virtue of their ability to bind to the IL-3 receptor complex while intrinsic activation properties of the ligand are diminished. IL-3, GM-CSF and or IL-5 also play a role in certain asthmatic responses.

An antagonist of the IL-3 receptor may have utility in this disease by blocking receptor-mediated activation and recruitment of inflammatory cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the human IL-3 gene for E. coli expression (pMON5873), encoding the polypeptide sequence of natural (wild type) human IL-3 [SEQ ID NO:128], plus an initiator methionine, as expressed in E. coli, with the amino acids numbered from the N-terminus of the natural hIL-3.

Figure 2: ClaI to NsiI Replacement Fragment. Figure 2 shows the nucleotide sequence of the replacement fragment used between the ClaI and NsiI sites of the hIL-3 gene. The codon choice used in the fragment corresponds to that found in highly expressed E. coli genes (Gouy and Gautier, 1982). Three new unique restriction sites, EcoRV, XhoI and PstI were introduced for the purpose of inserting synthetic gene fragments. The portion of the coding sequence shown encodes hIL-3 amino acids 20-70.

Figure 3 shows the nucleotide and amino acid sequence of the gene in pMON5873 with the sequence extending from NcoI through HindIII. The codon choices used to encode amino acids 1-14 and 107-133 correspond to that found in highly expressed E. coli genes.

Figure 4 shows the construction of the plasmid vector pMON5846 which encodes [Met-(1-133) hIL-3 (Arg^{129})].

Figure 5 shows the construction of the plasmid vector pMON5847 (ATCC 68912) which encodes [Met-(1-133) hIL-3 (Arg^{129})].

Figure 6 shows the construction of plasmid vector pMON5853 which encodes [Met-(15-133) hIL-3 (Arg^{129})].

Figure 7 shows the construction of the plasmid vector pMON5854 which encodes [Met-(1-133) hIL-3 (Arg^{129})].

Figure 8 shows the DNA sequence and resulting amino acid sequence of the lamB signal peptide.

Figure 9 shows the construction of the plasmid vector pMON5978 which encodes Met-Ala-(15-125)hIL-3.

Figure 10 shows the construction of the plasmid vector pMON5988 which encodes Met-Ala(15-125)hIL-3.

Figure 11 shows the construction of the plasmid vector pMON5887 which encodes Met-(1-125)hIL-3.

Figure 12 shows the construction of pMON6457 which encodes (15-125)hIL-3; it contains the araBAD promoter and the lamB signal peptide fused to the variant hIL-3 amino acids 15-125.

Figure 13 shows the construction of pMON6458; it contains the araBAD promoter and the lamB signal peptide fused to the variant hIL-3 amino acids 15-125.

Figure 14 shows the construction of pMON6467 in which the bases encoding amino acids 35-40 of hIL-3 were deleted using site-directed PCR mutagenesis methods. pMON6467 was used as the template for the generation of single amino acid variants at positions 35-40 of hIL-3.

Figure 15 shows the construction of single amino acid substitutions at position 35 of hIL-3 using site-directed PCR mutagenesis methods. The mutagenesis results in 20 different single amino substitutions, which is

referred to as a "library", at position 35 of hIL-3.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to muteins of human interleukin-3 (hIL-3) in which amino acid substitutions have been made at from one to three positions in the amino acid sequence of the polypeptide and to hIL-3 muteins which have substantially the same structure and substantially the same biological activity. Preferred muteins of the present invention are (15-125)hIL-3 deletion mutants which have deletions of amino acids 1 to 14 at the N-terminus and/or 126 to 133 at the C-terminus and which both also have from one to three amino acid substitutions in the polypeptide and muteins having substantially the same structure and substantially the same biological activity. As used herein human interleukin-3 corresponds to the amino acid sequence (1-133) as depicted in Figure 1 and (15-125) hIL-3 corresponds to the 15 to 125 amino acid sequence of the hIL-3 polypeptide. Naturally occurring variants of hIL-3 polypeptide amino acids are also included in the present invention (for example, the allele in which proline rather than serine is at position 8 in the hIL-3 polypeptide sequence) as are variant hIL-3 molecules which are modified post-translationally (e.g. glycosylation).

The present invention also includes the DNA sequences which code for the mutant polypeptides, DNA sequences which are substantially similar and perform substantially the same function, and DNA sequences which differ from the DNAs encoding the muteins of the invention only due to the degeneracy of the genetic code.

Included in the present invention are novel mutant human interleukin-3 polypeptides comprising a polypeptide having the amino acid sequence of native human

interleukin-3 wherein amino acids 126 to 133 have been deleted from the C-terminus of the native human interleukin-3 polypeptide and amino acids 1 to 14 have been deleted from the N-terminus of the native human interleukin-3 polypeptide and, in addition, polypeptides of the present invention also have one to three amino acid substitutions in the polypeptide sequence. The muteins of the present invention can have from one to three amino acid substitutions in the hIL-3 polypeptide chain and, in addition, can have deletions of amino acids at the N-terminus and/or the C-terminus.

Also included in the present invention are the DNA sequences coding for the muteins of the present invention; the oligonucleotide intermediates used to construct the mutant DNAs; and the polypeptides coded for by these oligonucleotides. These polypeptides may be useful as antagonists or as antigenic fragments for the production of antibodies useful in immunoassay and immunotherapy protocols.

The mutant hIL-3 polypeptides of the present invention may also have methionine, alanine, or methionine-alanine residues inserted at the N-terminus.

The present invention includes hIL-3 mutant polypeptides of the formula I:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
80 85 90

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
95 100 105

Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:15]
125 130

wherein Xaa at position 17 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;

Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;

Xaa at position 19 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;

Xaa at position 20 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;

Xaa at position 21 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn, Thr, Ser or Val;

Xaa at position 22 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln, Leu, Val or Gly;

Xaa at position 23 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe, Leu, Ser, or Arg;

Xaa at position 24 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;

Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;

Xaa at position 26 is His, Thr, Phe, Gly, Arg, Ala, or Trp;

Xaa at position 27 is Leu, Gly, Arg, Thr, Ser, or Ala;

Xaa at position 28 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;

Xaa at position 29 is Gln, Asn, Leu, Pro, Arg, or Val;

Xaa at position 30 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;

Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;

Xaa at position 32 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 33 is Pro, Leu, Gln, Ala, Thr, or Glu;

Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr, Arg, Ala, Phe, Ile or Met;

Xaa at position 35 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;

Xaa at position 36 is Asp, Leu, or Val;

Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;

Xaa at position 38 is Asn, or Ala;

Xaa at position 40 is Leu, Trp, or Arg;

Xaa at position 41 is Asn, Cys, Arg, Leu, His, Met, or Pro;

Xaa at position 42 is Gly, Asp, Ser, Cys, Asn, Lys, Thr, Leu, Val, Glu, Phe, Tyr, Ile, Met or Ala;

Xaa at position 43 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, Gln, Arg, Thr, Gly or Ser;

Xaa at position 44 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, Glu, Asn, Gln, Ala or Pro;

Xaa at position 45 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, Trp, Asp, Asn, Arg, Ser, Ala, Ile, Glu or His;

Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln, Lys, His, Ala, Tyr, Ile, Val or Gly;

Xaa at position 47 is Ile, Gly, Val, Ser, Arg, Pro, or His;

Xaa at position 48 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu, Lys, Thr, Ala, Met, Val or Asn;

Xaa at position 49 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;

Xaa at position 50 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser, Ala, Ile, Val, His, Phe, Met or Gln;

Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;

Xaa at position 52 is Asn, His, Arg, Leu, Gly, Ser, or Thr;

Xaa at position 53 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser, or Met;

Xaa at position 54 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn, Lys, His, Ala or Leu;

Xaa at position 55 is Arg, Thr, Val, Ser, Leu, or Gly;

Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His, Thr, Ala, Tyr, Phe, Leu, Val or Lys;

Xaa at position 57 is Asn or Gly;

Xaa at position 58 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;

Xaa at position 59 is Glu Tyr, His, Leu, Pro, or Arg;

Xaa at position 60 is Ala, Ser, Pro, Tyr, Asn, or Thr;

Xaa at position 61 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
Xaa at position 62 is Asn His, Val, Arg, Pro, Thr, Asp, or Ile;
Xaa at position 63 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or Val;
Xaa at position 64 is Ala, Asn, Pro, Ser, or Lys;
Xaa at position 65 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 67 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or His;
Xaa at position 68 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;
Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or Leu;
Xaa at position 70 is Asn, Leu, Val, Trp, Pro, or Ala;
Xaa at position 71 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln, Trp, or Asn;
Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;
Xaa at position 74 is Ile, Met, Thr, Pro, Arg, Gly, Ala;
Xaa at position 75 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser, Gln, or Leu;
Xaa at position 76 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or Asp;
Xaa at position 77 is Ile, Ser, Arg, Thr, or Leu;
Xaa at position 78 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;
Xaa at position 79 is Lys, Thr, Asn, Met, Arg, Ile, Gly, or Asp;
Xaa at position 80 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 81 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;
Xaa at position 82 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn, His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;
Xaa at position 83 is Pro, Ala, Thr, Trp, Arg, or Met;
Xaa at position 84 is Cys, Glu, Gly, Arg, Met, or Val;
Xaa at position 85 is Leu, Asn, Val, or Gln;
Xaa at position 86 is Pro, Cys, Arg, Ala, or Lys;
Xaa at position 87 is Leu, Ser, Trp, or Gly;
Xaa at position 88 is Ala, Lys, Arg, Val, or Trp;
Xaa at position 89 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn, or Ser;
Xaa at position 90 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or Met;

Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;
Xaa at position 92 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile or Leu;
Xaa at position 93 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;
Xaa at position 94 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys, His, Ala, or Pro;
Xaa at position 95 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr, Asn, Lys, Ser, Ala, Trp, Phe, Ile, or Tyr;
Xaa at position 96 is Pro, Lys, Tyr, Gly, Ile, or Thr;
Xaa at position 97 is Ile, Val, Lys, Ala, or Asn;
Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr, Glu, Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;
Xaa at position 99 is Ile, Leu, Arg, Asp, Val, Pro, Gln, Gly, Ser, Phe, or His;
Xaa at position 100 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln, or Pro;
Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val, Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu, or Gln;
Xaa at position 102 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
Xaa at position 103 is Asp, or Ser;
Xaa at position 104 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu, Gln, Lys, Ala, Phe, or Gly;
Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
Xaa at position 106 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;
Xaa at position 108 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His, Ser, Ala or Pro;
Xaa at position 109 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;
Xaa at position 110 is Lys, Ala, Asn, Thr, Leu, Arg, Gln, His, Glu, Ser, Ala, or Trp;
Xaa at position 111 is Leu, Ile, Arg, Asp, or Met;
Xaa at position 112 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;
Xaa at position 113 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp, Lys, Leu, Ile, Val or Asn;
Xaa at position 114 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;
Xaa at position 115 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr, Trp, or Met;
Xaa at position 116 is Lys, Leu, Pro, Thr, Met, Asp, Val, Glu,

Arg, Trp, Ser, Asn, His, Ala, Tyr, Phe, Gln, or Ile;
Xaa at position 117 is Thr, Ser, Asn, Ile, Trp, Lys, or Pro;
Xaa at position 118 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;
Xaa at position 119 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;
Xaa at position 120 is Asn, Ala, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or Gly;
Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;
and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3 with the proviso that when Xaa at position 22 is Leu, and/or Xaa at position 34 is Gly or Glu, and/or Xaa at position 44 is Ala, and/or Xaa at position 46 is Lys or Ala, and/or Xaa at position 50 is Lys, and/or Xaa at position 59 is Pro or Arg, and/or Xaa at position 63 is Lys, and/or Xaa at position 75 is Gly or Arg, and/or Xaa at position 94 is Pro, and/or Xaa at position 98 is Arg, and/or Xaa at position 106 is Lys, and/or Xaa at position 110 is Ala or Glu, and/or Xaa at position 111 is Met, then there must be at least one additional substitution besides the ones indicated.

Included in the present invention are (1-133)hIL-3 mutant polypeptides of the Formula II:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Xaa Xaa Xaa Xaa Xaa Glu Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa Xaa Glu Xaa Xaa
35 40 45

Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa Xaa

50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75

Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Cys Xaa Pro Xaa Xaa Xaa Xaa
80 85 90

Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa Xaa
95 100 105

Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Xaa Xaa
110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:16]
125 130

wherein

Xaa at position 17 is Ser, Gly, Asp, Met, or Gln;

Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;

Xaa at position 19 is Met, Phe, Ile, Arg, or Ala;

Xaa at position 20 is Ile or Pro;

Xaa at position 21 is Asp or Glu;

Xaa at position 23 is Ile, Val, Ala, Leu, or Gly;

Xaa at position 24 is Ile, Val, Phe, or Leu;

Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;

Xaa at position 26 is His, Phe, Gly, Arg, or Ala;

Xaa at position 28 is Lys, Leu, Gln, Gly, Pro, or Val;

Xaa at position 29 is Gln, Asn, Leu, Arg, or Val;

Xaa at position 30 is Pro, His, Thr, Gly, or Gln;

Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;

Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 33 is Pro, Leu, Gln, Ala, or Glu;

Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln, Glu, Ile, Phe, Thr or Met;

Xaa at position 35 is Leu, Ala, Asn, Pro, Gln, or Val;

Xaa at position 36 is Asp or Leu;

Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;

Xaa at position 38 is Asn or Ala;

Xaa at position 41 is Asn, Cys, Arg, His, Met, or Pro;

Xaa at position 42 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu, Met, Tyr, Val or Arg;

Xaa at position 44 is Asp or Glu;

Xaa at position 45 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn, Glu, Ser, or Trp;

Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln, Glu, His, Ile, Lys, Tyr, Val or Gly;

Xaa at position 47 is Ile, Val, or His;

Xaa at position 49 is Met, Asn, or Asp;

Xaa at position 50 is Glu, Thr, Ala, Asn, Ser or Asp;

Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;

Xaa at position 52 is Asn or Gly;

Xaa at position 53 is Leu, Met, or Phe;

Xaa at position 54 is Arg, Ala, or Ser;

Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;

Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn, Glu, His, Leu, Thr, Val or Lys;

Xaa at position 59 is Glu, Tyr, His, Leu, or Arg;

Xaa at position 60 is Ala, Ser, Asn, or Thr;

Xaa at position 61 is Phe or Ser;

Xaa at position 62 is Asn, Val, Pro, Thr, or Ile;

Xaa at position 63 is Arg, Tyr, Lys, Ser, His, or Val;

Xaa at position 64 is Ala or Asn;

Xaa at position 65 is Val, Thr, Leu, or Ser;

Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;

Xaa at position 67 is Ser, Phe, Val, Gly, Asn, Ile, or His;

Xaa at position 68 is Leu, Val, Ile, Phe, or His;

Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;

Xaa at position 70 is Asn or Pro;

Xaa at position 71 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;

Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;

Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or Pro;

Xaa at position 74 is Ile or Met;

Xaa at position 75 is Glu, Gly, Asp, Ser, or Gln;

Xaa at position 76 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or

Asp;

Xaa at position 77 is Ile, Ser, or Leu;

Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or Asp;

Xaa at position 80 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;

Xaa at position 81 is Leu, or Val;

Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His, Met, Phe, Ser, Thr, Tyr or Val;

Xaa at position 83 is Pro, Ala, Thr, Trp, or Met;

Xaa at position 85 is Leu or Val;

Xaa at position 87 is Leu or Ser;

Xaa at position 88 is Ala, Arg, or Trp;

Xaa at position 89 is Thr, Asp, Glu, His, Asn, or Ser;

Xaa at position 90 is Ala, Asp, or Met;

Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;

Xaa at position 92 is Pro or Ser;

Xaa at position 93 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;

Xaa at position 95 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile, Phe, Ser or Thr;

Xaa at position 96 is Pro or Tyr;

Xaa at position 97 is Ile, Val, or Ala;

Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr, Leu, Arg, Gln, Glu, lys, Met, Ser, Tyr, Val or Pro;

Xaa at position 99 is Ile, Leu, Val, or Phe;

Xaa at position 100 is Lys, Leu, His, Arg, Ile, Gln, Pro, or Ser;

Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val, Asn, Ile, Leu or Tyr;

Xaa at position 102 is Gly, Glu, Lys, or Ser;

Xaa at position 104 is Trp, Val, Tyr, Met, or Leu;

Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;

Xaa at position 106 is Glu, Ser, Ala, or Gly;

Xaa at position 108 is Arg, Ala, Gln, Ser or Lys;

Xaa at position 109 is Arg, Thr, Glu, Leu, Ser, or Gly;

Xaa at position 112 is Thr, Val, Gln, Glu, His, or Ser;

Xaa at position 114 is Tyr or Trp;

Xaa at position 115 is Leu or Ala;

Xaa at position 116 is Lys, Thr, Met, Val, Trp, Ser, Leu, Ala, Asn, Gln, His, Met, Phe, Tyr or Ile;
Xaa at position 117 is Thr, Ser, or Asn;
Xaa at position 119 is Glu, Ser, Pro, Leu, Thr, or Tyr;
Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or Gly;
Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3 with the proviso that when Xaa at position 34 is Gly or/and Xaa or position 46 is Lys or Ala or/and Xaa at position 59 is Arg and/or Xaa at position 63 is Lys and/or Xaa at position 75 is Gly and/or Xaa at position 98 is Arg then there must be at least one additional substitution besides the ones indicated.

Included in the present invention are (1-133)hIL-3 mutant polypeptides of the Formula III:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Xaa Xaa Xaa Ile Xaa Glu Xaa Xaa Xaa Xaa Leu Lys Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Asp Xaa Xaa Asn Leu Asn Xaa Glu Xaa Xaa
35 40 45

Xaa Ile Leu Met Xaa Xaa Asn Leu Xaa Xaa Xaa Asn Leu Glu Xaa
50 55 60

Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Ile Glu
65 70 75

Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Cys Xaa Pro Xaa Xaa Thr Ala
80 85 90

Xaa Pro Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Asp Xaa Xaa
95 100 105

Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Glu Xaa
110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:17]
125 130

wherein

Xaa at position 17 is Ser, Gly, Asp, Met, or Gln;

Xaa at position 18 is Asn, His, or Ile;

Xaa at position 19 is Met or Ile;

Xaa at position 21 is Asp or Glu;

Xaa at position 23 is Ile, Ala, Leu, or Gly;

Xaa at position 24 is Ile, Val, or Leu;

Xaa at position 25 is Thr, His, Gln, or Ala;

Xaa at position 26 is His or Ala;

Xaa at position 29 is Gln, Asn, or Val;

Xaa at position 30 is Pro, Gly, or Gln;

Xaa at position 31 is Pro, Asp, Gly, or Gln;

Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 33 is Pro or Glu;

Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln, Glu, Ile, Phe, Thr or Met;

Xaa at position 35 is Leu, Ala, Asn, Pro, Gln, or Val;

Xaa at position 37 is Phe, Ser, Pro, or Trp;

Xaa at position 38 is Asn or Ala;

Xaa at position 42 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu, Met, Tyr or Arg;

Xaa at position 44 is Asp or Glu;

Xaa at position 45 is Gln, Val, Met, Leu, Thr, Ala, Asn, Glu, Ser or Lys;

Xaa at position 46 is Asp, Phe, Ser, Thr, Ala, Asn Gln, Glu, His, Ile, Lys, Tyr, Val or Cys;

Xaa at position 50 is Glu, Ala, Asn, Ser or Asp;

Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;

Xaa at position 54 is Arg or Ala;

Xaa at position 54 is Arg or Ala;

Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;

Xaa at position 56 is Pro, Gly, Ser, Gln, Ala, Arg, Asn, Glu, Leu, Thr, Val or Lys;

Xaa at position 60 is Ala or Ser;

Xaa at position 62 is Asn, Pro, Thr, or Ile;

Xaa at position 63 is Arg or Lys;

Xaa at position 64 is Ala or Asn;

Xaa at position 65 is Val or Thr;

Xaa at position 66 is Lys or Arg;

Xaa at position 67 is Ser, Phe, or His;

Xaa at position 68 is Leu, Ile, Phe, or His;

Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;

Xaa at position 71 is Ala, Pro, or Arg;

Xaa at position 72 is Ser, Glu, Arg, or Asp;

Xaa at position 73 is Ala or Leu;

Xaa at position 76 is Ser, Val, Ala, Asn, Glu, Pro, or Gly;

Xaa at position 77 is Ile or Leu;

Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or Asp;

Xaa at position 80 is Asn, Gly, Glu, or Arg;

Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His, Ile, Met, Phe, Ser, Thr, Tyr or Val;

Xaa at position 83 is Pro or Thr;

Xaa at position 85 is Leu or Val;

Xaa at position 87 is Leu or Ser;

Xaa at position 88 is Ala or Trp;

Xaa at position 91 is Ala or Pro;

Xaa at position 93 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;

Xaa at position 95 is His, Pro, Arg, Val, Leu, Gly, Asn, Phe, Ser or Thr;

Xaa at position 96 is Pro or Tyr;

Xaa at position 97 is Ile or Val;

Xaa at position 98 is His, Ile, Asn, Leu, Ala, Thr, Leu, Arg, Gln, Leu, Lys, Met, Ser, Tyr, Val or Pro;

Xaa at position 99 is Ile, Leu, or Val;

Xaa at position 100 is Lys, Arg, Ile, Gln, Pro, or Ser;

Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Pro, Asn, Ile, Leu or Tyr;

Xaa at position 104 is Trp or Leu;

Xaa at position 105 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;

Xaa at position 106 is Glu or Gly;

Xaa at position 108 is Arg, Ala, or Ser;

Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;

Xaa at position 112 is Thr, Val, or Gln;

Xaa at position 114 is Tyr or Trp;

Xaa at position 115 is Leu or Ala;

Xaa at position 116 is Lys, Thr, Val, Trp, Ser, Ala, His, Met, Phe, Tyr or Ile;

Xaa at position 117 is Thr or Ser;

Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;

Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;

Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;

Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3 with the proviso that when Xaa at position 22 is Leu, and/or Xaa at position 34 is Gly or Glu, and/or Xaa at position 44 is Ala, and/or Xaa at position 46 is Lys or Ala, and/or Xaa at position 50 is Lys, and/or Xaa at position 59 is Pro or Arg, and/or Xaa at position 63 is Lys, and/or Xaa at position 75 is Gly or Arg, and/or Xaa at position 94 is Pro, and/or Xaa at position 98 is Arg, and/or Xaa at position 106 is Lys, and/or Xaa at position 110 is Ala or Glu, and/or Xaa at position 111 is Met, then there must be at least one additional substitution besides the ones indicated.

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133)human interleukin-3 with the proviso that when Xaa at position 34 is Gly and/or Xaa at position 46 is Lys or Ala, and/or Xaa at position 63 is Lys, and/or Xaa at position 98 is Arg, then two or three of the amino acid designated by Xaa are different from the corresponding amino acids of the native (1-133) human interleukin-3.

Included in the present invention are (1-133)hIL-3 mutant polypeptides of the Formula IV:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Xaa Xaa Met Ile Asp Glu Xaa Ile Xaa Xaa Leu Lys Xaa Xaa
20 25 30

Pro Xaa Pro Xaa Xaa Asp Phe Xaa Asn Leu Asn Xaa Glu Asp Xaa
35 40 45

Xaa Ile Leu Met Xaa Xaa Asn Leu Arg Xaa Xaa Asn Leu Glu Ala
50 55 60

Phe Xaa Arg Xaa Xaa Lys Xaa Xaa Xaa Asn Ala Ser Ala Ile Glu
65 70 75

Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Cys Leu Pro Xaa Xaa Thr Ala
80 85 90

Xaa Pro Xaa Arg Xaa Pro Ile Xaa Xaa Xaa Xaa Gly Asp Trp Xaa
95 100 105

Glu Phe Xaa Xaa Lys Leu Xaa Phe Tyr Leu Xaa Xaa Leu Glu Xaa

110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:18]

125 130

wherein

Xaa at position 17 is Ser, Gly, Asp, or Gln;

Xaa at position 18 is Asn, His, or Ile;

Xaa at position 23 is Ile, Ala, Leu, or Gly;

Xaa at position 25 is Thr, His, or Gln;

Xaa at position 26 is His or Ala;

Xaa at position 29 is Gln or Asn;

Xaa at position 30 is Pro or Gly;

Xaa at position 32 is Leu, Arg, Asn, or Ala;

Xaa at position 34 is Leu, Val, Ser, Ala, Arg, Gln, Glu, Ile, Phe, Thr, or Met;

Xaa at position 35 is Leu, Ala, Asn, or Pro;

Xaa at position 38 is Asn or Ala;

Xaa at position 42 is Gly, Asp, Ser, Ala, Asn, Ile, Leu, Met, Tyr or Arg;

Xaa at position 45 is Gln, Val, Met, Leu, Ala, Asn, Glu, or Lys;

Xaa at position 46 is Asp, Phe, Ser, Ala, Gln, Glu, His, Val or Thr;

Xaa at position 50 is Glu Asn, Ser or Asp;

Xaa at position 51 is Asn, Arg, Pro, Thr, or His;

Xaa at position 55 is Arg, Leu, or Gly;

Xaa at position 56 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;

Xaa at position 62 is Asn, Pro, or Thr;

Xaa at position 64 is Ala or Asn;

Xaa at position 65 is Val or Thr;

Xaa at position 67 is Ser or Phe;

Xaa at position 68 is Leu or Phe;

Xaa at position 69 is Gln, Ala, Glu, or Arg;

Xaa at position 76 is Ser, Val, Asn, Pro, or Gly;

Xaa at position 77 is Ile or Leu;

Xaa at position 79 is Lys, Gly, Asn, Met, Arg, Ile, or Gly;

Xaa at position 80 is Asn, Gly, Glu, or Arg;

Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His, Met, Phe, Ser, Thr, Tyr or Val;

Xaa at position 87 is Leu or Ser;
Xaa at position 88 is Ala or Trp;
Xaa at position 91 is Ala or Pro;
Xaa at position 93 is Thr, Asp, or Ala;
Xaa at position 95 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;
Xaa at position 98 is His, Ile, Asn, Ala, Thr, Arg, Gln, Glu, Lys, Met, Ser, Tyr, Val or Leu;
Xaa at position 99 is Ile or Leu;
Xaa at position 100 is Lys or Arg;
Xaa at position 101 is Asp, Pro, Met, Lys, Thr, His, Pro, Asn, Ile, Leu or Tyr;
Xaa at position 105 is Asn, Pro, Ser, Ile or Asp;
Xaa at position 108 is Arg, Ala, or Ser;
Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;
Xaa at position 112 is Thr or Gln;
Xaa at position 116 is Lys, Val, Trp, Ala, His, Phe, Tyr or Ile;
Xaa at position 117 is Thr or Ser;
Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Pro, or Asp;
Xaa at position 122 is Gln, Met, Trp, Phe, Pro, His, Ile, or Tyr;
Xaa at position 123 is Ala, Met, Glu, Ser, or Leu;

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133)human interleukin-3.

Preferred polypeptides of the present invention are (15-125)hIL-3 mutant polypeptides of the Formula V:

Asn Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
80 85 90

Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
95 100 105

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:19]
110

wherein

Xaa at position 3 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;

Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;

Xaa at position 5 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;

Xaa at position 6 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;

Xaa at position 7 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn, Thr, Ser or Val;

Xaa at position 8 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln, Leu, Val, or Gly;

Xaa at position 9 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe, Leu, Ser, or Arg;

Xaa at position 10 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;

Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;

Xaa at position 12 is His, Thr, Phe, Gly, Arg, Ala, or Trp;

Xaa at position 13 is Leu, Gly, Arg, Thr, Ser, or Ala;

Xaa at position 14 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;

Xaa at position 15 is Gln, Asn, Leu, Pro, Arg, or Val;

Xaa at position 16 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;

Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;

Xaa at position 18 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 19 is Pro, Leu, Gln, Ala, Thr, or Glu;
Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr, Arg, Ala, Phe, Ile or Met;
Xaa at position 21 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;
Xaa at position 22 is Asp, Leu, or Val;
Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;
Xaa at position 24 is Asn, or Ala;
Xaa at position 26 is Leu, Trp, or Arg;
Xaa at position 27 is Asn, Cys, Arg, Leu, His, Met, Pro;
Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Lys, Asn, Thr, Leu, Val, Glu, Phe, Tyr, Ile or Met;
Xaa at position 29 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, Gln, Arg, Thr, Gly or Ser;
Xaa at position 30 is Asp, Ser, Leu, Arg, Lys, Thr,Met, Trp, Glu, Asn, Gln, Ala or Pro;
Xaa at position 31 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, Asp, Asn, Arg, Ser, Ala, Ile, Glu, His or Trp;
Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln, Lys, His, Ala, Tyr, Ile, Val or Gly;
Xaa at position 33 is Ile, Gly, Val, Ser, Arg, Pro, or His;
Xaa at position 34 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu, Lys, Thr, Ala, Met, Val or Asn;
Xaa at position 35 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;
Xaa at position 36 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser, Ala, Ile, Val, His, Phe, Met or Gln;
Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 38 is Asn, His, Arg, Leu, Gly, Ser, or Thr;
Xaa at position 39 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser, Met, or;
Xaa at position 40 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn, Lys, His, Ala or Leu;
Xaa at position 41 is Arg, Thr, Val, Ser, Leu, or Gly;
Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His, Thr, Ala, Tyr, Phe, Leu, Val or Lys;
Xaa at position 43 is Asn or Gly;
Xaa at position 44 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
Xaa at position 45 is Glu Tyr, His, Leu, Pro, or Arg;
Xaa at position 46 is Ala, Ser, Pro, Tyr, Asn, or Thr;

Xaa at position 47 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
Xaa at position 48 is Asn, His, Val, Arg, Pro, Thr, Asp, or Ile;
Xaa at position 49 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or Val;
Xaa at position 50 is Ala, Asn, Pro, Ser, or Lys;
Xaa at position 51 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 53 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or His;
Xaa at position 54 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or Leu;
Xaa at position 56 is Asn, Leu, Val, Trp, Pro, or Ala;
Xaa at position 57 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln, Trp, or Asn;
Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;
Xaa at position 60 is Ile, Met, Thr, Pro, Arg, Gly, Ala;
Xaa at position 61 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser, Gln, or Leu;
Xaa at position 62 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or Asp;
Xaa at position 63 is Ile, Ser, Arg, Thr, or Leu;
Xaa at position 64 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;
Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or Asp;
Xaa at position 66 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 67 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;
Xaa at position 68 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn, His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;
Xaa at position 69 is Pro, Ala, Thr, Trp, Arg, or Met;
Xaa at position 70 is Cys, Glu, Gly, Arg, Met, or Val;
Xaa at position 71 is Leu, Asn, Val, or Gln;
Xaa at position 72 is Pro, Cys, Arg, Ala, or Lys;
Xaa at position 73 is Leu, Ser, Trp, or Gly;
Xaa at position 74 is Ala, Lys, Arg, Val, or Trp;
Xaa at position 75 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn, or Ser;
Xaa at position 76 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or Met;

Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;
Xaa at position 78 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile or Leu;
Xaa at position 79 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;
Xaa at position 80 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys, His, Ala or Pro;
Xaa at position 81 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr, Asn, Lys, Ser, Ala, Trp, Phe, Ile or Tyr;
Xaa at position 82 is Pro, Lys, Tyr, Gly, Ile, or Thr;
Xaa at position 83 is Ile, Val, Lys, Ala, or Asn;
Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr, Glu, Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;
Xaa at position 85 is Ile, Leu, Arg, Asp, Val, Pro, Gln, Gly, Ser, Phe, or His;
Xaa at position 86 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln, Pro;
Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val, Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu or Gln;
Xaa at position 88 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
Xaa at position 89 is Asp, or Ser;
Xaa at position 90 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu, Gln, Lys, Ala, Phe, or Gly;
Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
Xaa at position 92 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;
Xaa at position 94 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His, Ser, Ala, or Pro;
Xaa at position 95 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;
Xaa at position 96 is Lys, Asn, Thr, Leu, Gln, Arg, His, Glu, Ser, Ala or Trp;
Xaa at position 97 is Leu, Ile, Arg, Asp, or Met;
Xaa at position 98 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;
Xaa at position 99 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp, Lys, Leu, Ile, Val or Asn;
Xaa at position 100 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;
Xaa at position 101 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr, Trp, or Met;
Xaa at position 102 is Lys, Leu, Pro, Thr, Met, Asp, Val, Glu, Arg,

Trp, Ser, Asn, His, Ala, Tyr, Phe, Gln, or Ile;
Xaa at position 103 is Thr, Ser, Asn, Ile, Trp, Lys, or Pro;
Xaa at position 104 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;
Xaa at position 105 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;
Xaa at position 106 is Asn, Ala, Pro, Leu, His, Val, or Gln;
Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or Gly;
Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- or Met-Ala- preceding the amino acid in position 1; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding native amino acids of (1-133) human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

Included in the present invention are (15-125)hIL-3 mutant polypeptides of the Formula VI:

```
Asn Cys Xaa Xaa Xaa Xaa Xaa Glu Xaa Xaa Xaa Xaa Leu Xaa Xaa
 1               5                  10                  15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa Xaa Glu Xaa
            20                  25                  30

Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa
            35                  40                  45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            50                  55                  60

Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Cys Xaa Pro Xaa Xaa Xaa
            65                  70                  75

Xaa Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa
            80                  85                  90
```

Xaa Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Xaa
95 100 105

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:20]
110

wherein

Xaa at position 3 is Ser, Gly, Asp, Met, or Gln;

Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;

Xaa at position 5 is Met, Phe, Ile, Arg, or Ala;

Xaa at position 6 is Ile or Pro;

Xaa at position 7 is Asp, or Glu;

Xaa at position 9 is Ile, Val, Ala, Leu, or Gly;

Xaa at position 10 is Ile, Val, Phe, or Leu;

Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;

Xaa at position 12 is His, Phe, Gly, Arg, or Ala;

Xaa at position 14 is Lys, Leu, Gln, Gly, Pro, or Val;

Xaa at position 15 is Gln, Asn, Leu, Arg, or Val;

Xaa at position 16 is Pro, His, Thr, Gly, or Gln;

Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;

Xaa at position 18 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 19 is Pro, Leu, Gln, Ala, or Glu;

Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln, Glu, Ile, Phe, Thr or Met;

Xaa at position 21 is Leu, Ala, Asn, Pro, Gln, or Val;

Xaa at position 22 is Asp or Leu;

Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;

Xaa at position 24 is Asn or Ala;

Xaa at position 27 is Asn, Cys, Arg, His, Met, or Pro;

Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu, Met, Tyr, or Arg;

Xaa at position 30 is Asp, or Glu;

Xaa at position 31 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn Glu, Ser or Trp;

Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln, Glu, His, Ile, Lys, Tyr, Val or Gly;

Xaa at position 33 is Ile, Val, or His;

Xaa at position 35 is Met, Asn, or Asp;

Xaa at position 36 is Glu, Thr, Ala, Asn, Ser or Asp;
Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 38 is Asn or Gly;
Xaa at position 39 is Leu, Met, or Phe;
Xaa at position 40 is Arg, Ala or Ser;
Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;
Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn, Glu, His, Leu, Thr, Val or Lys;
Xaa at position 45 is Glu, Tyr, His, Leu, or Arg;
Xaa at position 46 is Ala, Ser, Asn, or Thr;
Xaa at position 47 is Phe or Ser;
Xaa at position 48 is Asn, Val, Pro, Thr, or Ile;
Xaa at position 49 is Arg, Tyr, Lys, Ser, His, or Val;
Xaa at position 50 is Ala or Asn;
Xaa at position 51 is Val, Thr, Leu, or Ser;
Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 53 is Ser, Phe, Val, Gly, Asn, Ile, or His;
Xaa at position 54 is Leu, Val, Ile, Phe, or His;
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
Xaa at position 56 is Asn or Pro;
Xaa at position 57 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;
Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or Pro;
Xaa at position 60 is Ile or Met;
Xaa at position 61 is Glu, Gly, Asp, Ser, or Gln;
Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or Asp;
Xaa at position 63 is Ile, Ser, or Leu;
Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or Asp;
Xaa at position 66 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 67 is Leu, or Val;
Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 69 is Pro, Ala, Thr, Trp, or Met;
Xaa at position 71 is Leu or Val;
Xaa at position 73 is Leu or Ser;

Xaa at position 74 is Ala, Arg, or Trp;
Xaa at position 75 is Thr, Asp, Glu, His, Asn, or Ser;
Xaa at position 76 is Ala, Asp, or Met;
Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;
Xaa at position 78 is Pro or Ser;
Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile, Phe, Ser or Thr;
Xaa at position 82 is Pro or Tyr;
Xaa at position 83 is Ile, Val, or Ala;
Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr, Arg, Gln, Glu, Lys, Met, Ser, Tyr, Val or Pro;
Xaa at position 85 is Ile, Leu, Val, or Phe;
Xaa at position 86 is Lys, Leu, His, Arg, Ile, Gln, Pro or Ser;
Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val, Asn, Ile, Leu or Tyr;
Xaa at position 88 is Gly, Glu, Lys, or Ser;
Xaa at position 90 is Trp, Val, Tyr, Met, or Leu;
Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
Xaa at position 92 is Glu, Ser, Ala, or Gly;
Xaa at position 94 is Arg, Ala, Gln, Ser or Lys;
Xaa at position 95 is Arg, Thr, Glu, Leu, Ser, or Gly;
Xaa at position 98 is Thr, Val, Gln, Glu, His, or Ser;
Xaa at position 100 is Tyr or Trp;
Xaa at position 101 is Leu or Ala;
Xaa at position 102 is Lys, Thr, Met, Val, Trp, Ser, Leu, Ala, Asn, Gln, His, Met, Phe, Tyr or Ile;
Xaa at position 103 is Thr, Ser, or Asn;
Xaa at position 105 is Glu, Ser, Pro, Leu, Thr, or Tyr;
Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or Gly;
Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- or Met-Ala- preceding the amino acid in position 1; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

Included in the present invention are (15-125)hIL-3 mutant polypeptides of the Formula VII:

```
Asn Cys Xaa Xaa Xaa Ile Xaa Glu Xaa Xaa Xaa Xaa Leu Lys Xaa
 1               5                  10                  15

Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa Xaa Asn Leu Asn Xaa Glu Xaa
                20                  25                  30

Xaa Xaa Ile Leu Met Xaa Xaa Asn Leu Xaa Xaa Xaa Asn Leu Glu
                35                  40                  45

Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Ile
                50                  55                  60

Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Cys Xaa Pro Xaa Xaa Thr
                65                  70                  75

Ala Xaa Pro Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Asp Xaa
                80                  85                  90

Xaa Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Glu
                95                 100                 105

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:21]
               110
```

wherein

Xaa at position 3 is Ser, Gly, Asp, Met, or Gln;

Xaa at position 4 is Asn, His, or Ile;

Xaa at position 5 is Met or Ile;

Xaa at position 7 is Asp or Glu;

Xaa at position 9 is Ile, Ala, Leu, or Gly;
Xaa at position 10 is Ile, Val, or Leu;
Xaa at position 11 is Thr, His, Gln, or Ala;
Xaa at position 12 is His or Ala;
Xaa at position 15 is Gln, Asn, or Val;
Xaa at position 16 is Pro, Gly, or Gln;
Xaa at position 17 is Pro, Asp, Gly, or Gln;
Xaa at position 18 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 19 is Pro or Glu;
Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln, Glu, Ile, Phe, Thr or Met;
Xaa at position 21 is Leu, Ala, Asn, Pro, Gln, or Val;
Xaa at position 23 is Phe, Ser, Pro, or Trp;
Xaa at position 24 is Asn or Ala;
Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu, Met Tyr or Arg;
Xaa at position 30 is Asp or Glu;
Xaa at position 31 is Gln, Val, Met, Leu, Thr, Ala, Asn, Glu, Ser or Lys;
Xaa at position 32 is Asp, Phe, Ser, Thr, Ala, Asn, Gln, Glu, His, Ile, Lys, Tyr, Val or Cys;
Xaa at position 36 is Glu, Ala, Asn, Ser or Asp;
Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 40 is Arg or Ala;
Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;
Xaa at position 42 is Pro, Gly, Ser, Gln, Ala, Arg, Asn, Glu, Leu, Thr, Val or Lys;
Xaa at position 46 is Ala or Ser;
Xaa at position 48 is Asn, Pro, Thr, or Ile;
Xaa at position 49 is Arg or Lys;
Xaa at position 50 is Ala or Asn;
Xaa at position 51 is Val or Thr;
Xaa at position 52 is Lys or Arg;
Xaa at position 53 is Ser, Phe, or His;
Xaa at position 54 is Leu, Ile, Phe, or His;
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
Xaa at position 57 is Ala, Pro, or Arg;
Xaa at position 58 is Ser, Glu, Arg, or Asp;

Xaa at position 59 is Ala or Leu;

Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, or Gly;

Xaa at position 63 is Ile or Leu;

Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or Asp;

Xaa at position 66 is Asn, Gly, Glu, or Arg;

Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His, Ile, Met, Phe, Ser, Thr, Tyr or Val;

Xaa at position 69 is Pro or Thr;

Xaa at position 71 is Leu or Val;

Xaa at position 73 is Leu or Ser;

Xaa at position 74 is Ala or Trp;

Xaa at position 77 is Ala or Pro;

Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;

Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Phe, Ser or Thr;

Xaa at position 82 is Pro or Tyr;

Xaa at position 83 is Ile or Val;

Xaa at position 84 is His, Ile, Asn, Leu, Ala, Thr, Leu, Arg, Gln, Leu, Lys, Met, Ser, Tyr, Val or Pro;

Xaa at position 85 is Ile, Leu, or Val;

Xaa at position 86 is Lys, Arg, Ile, Gln, Pro, or Ser;

Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Asn, Ile, Leu or Tyr;

Xaa at position 90 is Trp or Leu;

Xaa at position 91 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;

Xaa at position 92 is Glu, or Gly;

Xaa at position 94 is Arg, Ala, or Ser;

Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;

Xaa at position 98 is Thr, Val, or Gln;

Xaa at position 100 is Tyr or Trp;

Xaa at position 101 is Leu or Ala;

Xaa at position 102 is Lys, Thr, Val, Trp, Ser, Ala, His, Met, Phe, Tyr or Ile;

Xaa at position 103 is Thr or Ser;

Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;

Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;

Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;

Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

which can additionally have Met- or Met-Ala- preceding the amino acid in position 1; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (15-125)human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

Included in the present invention are (15-125)hIL-3 mutant polypeptides of the Formula VIII:

Asn Cys Xaa Xaa Met Ile Asp Glu Xaa Ile Xaa Xaa Leu Lys Xaa
1 5 10 15

Xaa Pro Xaa Pro Xaa Xaa Asp Phe Xaa Asn Leu Asn Xaa Glu Asp
20 25 30

Xaa Xaa Ile Leu Met Xaa Xaa Asn Leu Arg Xaa Xaa Asn Leu Glu
35 40 45

Ala Phe Xaa Arg Xaa Xaa Lys Xaa Xaa Xaa Asn Ala Ser Ala Ile
50 55 60

Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Cys Leu Pro Xaa Xaa Thr
65 70 75

Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Xaa Xaa Xaa Gly Asp Trp
80 85 90

Xaa Glu Phe Xaa Xaa Lys Leu Xaa Phe Tyr Leu Xaa Xaa Leu Glu
95 100 105

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:22]
110

wherein

Xaa at position 3 is Ser, Gly, Asp, or Gln;

Xaa at position 4 is Asn, His, or Ile;

Xaa at position 9 is Ile, Ala, Leu, or Gly;

Xaa at position 11 is Thr, His, or Gln;

Xaa at position 12 is His or Ala;

Xaa at position 15 is Gln or Asn;

Xaa at position 16 is Pro or Gly;

Xaa at position 18 is Leu, Arg, Asn, or Ala;

Xaa at position 20 is Leu, Val, Ser, Ala, Arg, Gln, Glu, Ile, Phe, Thr or Met;

Xaa at position 21 is Leu, Ala, Asn, or Pro;

Xaa at position 24 is Asn or Ala;

Xaa at position 28 is Gly, Asp, Ser, Ala, Asn, Ile, Leu, Met, Tyr or Arg;

Xaa at position 31 is Gln, Val, Met, Leu, Ala, Asn, Glu or Lys;

Xaa at position 32 is Asp, Phe, Ser, Ala, Gln, Glu, His, Val or Thr;

Xaa at position 36 is Glu, Asn, Ser or Asp;

Xaa at position 37 is Asn, Arg, Pro, Thr, or His;

Xaa at position 41 is Arg, Leu, or Gly;

Xaa at position 42 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;

Xaa at position 48 is Asn, Pro, or Thr;

Xaa at position 50 is Ala or Asn;

Xaa at position 51 is Val or Thr;

Xaa at position 53 is Ser or Phe;

Xaa at position 54 is Leu or Phe;

Xaa at position 55 is Gln, Ala, Glu, or Arg;

Xaa at position 62 is Ser, Val, Asn, Pro, or Gly;

Xaa at position 63 is Ile or Leu;

Xaa at position 65 is Lys, Asn, Met, Arg, Ile, or Gly;

Xaa at position 66 is Asn, Gly, Glu, or Arg;

Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His, Met, Phe, Ser, Thr, Tyr or Val;

Xaa at position 73 is Leu or Ser;

Xaa at position 74 is Ala or Trp;

Xaa at position 77 is Ala or Pro;

Xaa at position 79 is Thr, Asp, or Ala;

Xaa at position 81 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;
Xaa at position 84 is His, Ile, Asn, Ala, Thr, Arg, Gln, Glu, Lys, Met, Ser, Tyr, Val or Leu;
Xaa at position 85 is Ile or Leu;
Xaa at position 86 is Lys or Arg;
Xaa at position 87 is Asp, Pro, Met, Lys, His, Pro, Asn, Ile, Leu or Tyr;
Xaa at position 91 is Asn, Pro, Ser, Ile or Asp;
Xaa at position 94 is Arg, Ala, or Ser;
Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;
Xaa at position 98 is Thr or Gln;
Xaa at position 102 is Lys, Val, Trp, or Ile;
Xaa at position 103 is Thr, Ala, His, Phe, Tyr or Ser;
Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 107 is Ala, Ser, Ile, Pro, or Asp;
Xaa at position 108 is Gln, Met, Trp, Phe, Pro, His, Ile, or Tyr;
Xaa at position 109 is Ala, Met, Glu, Ser, or Leu;

and which can additionally have Met- or Met-Ala- preceding the amino acid in position 1; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133)human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

In Formulas V, VI, VII and VIII the Asn in position 1 corresponds to the Asn in position 15 of native hIL-3 and positions 1 to 111 correspond to positions 15 to 125 in the native hIL-3 sequence shown in Figure 1.

Also included in the present invention are polypeptides of the following formula (IX):

1 5 10
$(Met)_m$-Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr
15 20
Ser Trp Val Asn Cys Ser Xaa Met Ile Asp Glu Ile Ile
25 30 35
Xaa His Leu Lys Xaa Pro Pro Xaa Pro Leu Leu Asp Xaa

40

40 45 50

Asn Asn Leu Asn Xaa Glu Asp Xaa Asp Ile Leu Met Glu

55 60

Xaa Asn Leu Arg Xaa Pro Asn Leu Xaa Xaa Phe Xaa Arg

65 70 75

Ala Val Lys Xaa Leu Xaa Asn Ala Ser Xaa Ile Glu Xaa

80 85

Ile Leu Xaa Asn Leu Xaa Pro Cys Leu Pro Xaa Ala Thr

90 95 100

Ala Ala Pro Xaa Arg His Pro Ile Xaa Ile Lys Xaa Gly

105 110 115

Asp Trp Xaa Glu Phe Arg Xaa Lys Leu Thr Phe Tyr Leu

120 125

Xaa Thr Leu Glu Xaa Ala Gln Xaa Gln Gln Thr Thr Leu

130

Ser Leu Ala Ile Phe [SEQ ID NO:129]

wherein m is 0 or 1; Xaa at position 18 is Asn or Ile; Xaa at position 25 is Thr or His; Xaa at position 29 is Gln, Arg, or Val; Xaa at position 32 is Leu, Ala, or Asn; Xaa at position 37 is Phe, Pro, or Ser; Xaa at position 42 is Glu, Ala, or Ser; Xaa at position 45 is Gln, Val, or Met; Xaa at position 51 is Asn or Arg; Xaa at position 55 is Arg, Leu, or Thr; Xaa at position 59 is Glu or Leu; Xaa at position 60 is Ala or Ser; Xaa at position 62 is Asn or Val; Xaa at position 67 is Ser, Asn, or His; Xaa at position 69 is Gln or Glu; Xaa at position 73 is Ala or Gly; Xaa at position 76 is Ser or Ala; Xaa at position 79 is Lys or Arg; Xaa at position 82 is Leu, Glu, or Val; Xaa at position 87 is Leu or Ser; Xaa at position 93 is Pro or Ser; Xaa at position 98 is His, Ile, or Thr; Xaa at position 101 is Asp or Ala; Xaa at position 105 is Asn or Glu; Xaa at position 109 is Arg or Glu; Xaa at position 116 is Lys or Val; Xaa at position 120 is Asn, Gln, or His; Xaa at position 123 is Ala or Glu; wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native human interleukin-3; or a polypeptide having

substantially the same structure and substantially the same biological activity.

Polypeptides of the present invention include those (15-125)hIL-3 muteins of the following formula (X):

1 5 10
$(Met_m\text{-}Ala_n)_p$-Asn Cys Ser Xaa Met Ile Asp Glu Ile Ile
15 20
Xaa His Leu Lys Xaa Pro Pro Xaa Pro Leu Leu Asp Xaa
25 30 35
Asn Asn Leu Asn Xaa Glu Asp Xaa Asp Ile Leu Met Glu
40 45
Xaa Asn Leu Arg Xaa Pro Asn Leu Xaa Xaa Phe Xaa Arg
50 55 60
Ala Val Lys Xaa Leu Xaa Asn Ala Ser Xaa Ile Glu Xaa
65 70 75
Ile Leu Xaa Asn Leu Xaa Pro Cys Leu Pro Xaa Ala Thr
80 85
Ala Ala Pro Xaa Arg His Pro Ile Xaa Ile Lys Xaa Gly
90 95 100
Asp Trp Xaa Glu Phe Arg Xaa Lys Leu Thr Phe Tyr Leu
105 110
Xaa Thr Leu Glu Xaa Ala Gln Xaa Gln Gln [SEQ ID NO:130]

wherein m is 0 or 1; n is 0 or 1; p is 0 or 1; Xaa at position 4 is Asn or Ile; Xaa at position 11 is Thr or His; Xaa at position 15 is Gln, Arg, or Val; Xaa at position 18 is Leu, Ala, or Asn; Xaa at position 23 is Phe, Pro, or Ser; Xaa at position 28 is Glu, Ala, or Ser; Xaa at position 31 is Gln, Val, or Met; Xaa at position 37 is Asn or Arg; Xaa at position 41 is Arg, Leu, or Thr; Xaa at position 45 is Glu or Leu; Xaa at position 46 is Ala or Ser; Xaa at position 48 is Asn or Val; Xaa at position 53 is Ser, Asn, or His; Xaa at position 55 is Gln or Glu; Xaa at position 59 is Ala or Gly; Xaa at position 62 is Ser or Ala; Xaa at position 65 is Lys or Arg; Xaa at position 68 is Leu, Glu, or Val; Xaa at position 73 is Leu or Ser; Xaa at position 79 is Pro or

Ser; Xaa at position 84 is His, Ile, or Thr; Xaa at position 87 is Asp or Ala; Xaa at position 91 is Asn or Glu; Xaa at position 95 is Arg or Glu; Xaa at position 102 is Lys or Val; Xaa at position 106 is Asn, Gln, or His; Xaa at position 109 is Ala or Glu;

wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (15-125)human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

The present invention includes polypeptides of Formula IX and Formula X above wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native human interleukin-3 or native (15-125) human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

"Mutant amino acid sequence," "mutant protein" or "mutant polypeptide" refers to a polypeptide having an amino acid sequence which varies from a native sequence or is encoded by a nucleotide sequence intentionally made variant from a native sequence. "Mutant protein," "variant protein" or "mutein" means a protein comprising a mutant amino acid sequence and includes polypeptides which differ from the amino acid sequence of native hIL-3 due to amino acid deletions, substitutions, or both. "Native sequence" refers to an amino acid or nucleic acid sequence which is identical to a wild-type or native form of a gene or protein.

Human IL-3 can be characterized by its ability to stimulate colony formation by human hematopoietic progenitor cells. The colonies formed include erythroid, granulocyte, megakaryocyte, granulocytic macrophages and mixtures thereof. Human IL-3 has demonstrated an ability

to restore bone marrow function and peripheral blood cell populations to therapeutically beneficial levels in studies performed initially in primates and subsequently in humans (Gillio, A. P., et al. (1990); Ganser, A., et al. (1990); Falk, S., et al. (1991). Additional activities of hIL-3 include the ability to stimulate leukocyte migration and chemotaxis; the ability to prime human leukocytes to produce high levels of inflammatory mediators like leukotrienes and histamine; the ability to induce cell surface expression of molecules needed for leukocyte adhesion; and the ability to trigger dermal inflammatory responses and fever. Many or all of these biological activities of hIL-3 involve signal transduction and high affinity receptor binding. Mutant polypeptides of the present invention may exhibit useful properties such as having similar or greater biological activity when compared to native hIL-3 or by having improved half-life or decreased adverse side effects, or a combination of these properties. They may also be useful as antagonists. hIL-3 mutant polypeptides which have little or no activity when compared to native hIL-3 may still be useful as antagonists, as antigens for the production of antibodies for use in immunology or immunotherapy, as genetic probes or as intermediates used to construct other useful hIL-3 muteins. Since hIL-3 functions by binding to its receptor(s) and triggering second messages resulting in competent signal transduction, hIL-3 muteins of this invention may be useful in helping to determine which specific amino acid sequences are responsible for these activities.

The novel hIL-3 mutant polypeptides of the present invention will preferably have at least one biological property of human IL-3 or of an IL-3-like growth factor and may have more than one IL-3-like biological property, or an improved property, or a reduction in an undesirable biological property of human IL-3. Some mutant polypeptides of the present invention may also exhibit an

improved side effect profile. For example, they may exhibit a decrease in leukotriene release or histamine release when compared to native hIL-3 or (15-125) hIL-3. Such hIL-3 or hIL-3-like biological properties may include one or more of the following biological characteristics and in vivo and in vitro activities.

One such property is the support of the growth and differentiation of progenitor cells committed to erythroid, lymphoid, and myeloid lineages. For example, in a standard human bone marrow assay, an IL-3-like biological property is the stimulation of granulocytic type colonies, megakaryocytic type colonies, monocyte/macrophage type colonies, and erythroid bursts. Other IL-3-like properties are the interaction with early multipotential stem cells, the sustaining of the growth of pluripotent precursor cells, the ability to stimulate chronic myelogenous leukemia (CML) cell proliferation, the stimulation of proliferation of mast cells, the ability to support the growth of various factor-dependent cell lines, and the ability to trigger immature bone marrow cell progenitors. Other biological properties of IL-3 have been disclosed in the art. Human IL-3 also has some biological activities which may in some cases be undesirable, for example the ability to stimulate leukotriene release and the ability to stimulate increased histamine synthesis in spleen and bone marrow cultures and in vivo.

Biological activity of hIL-3 and hIL-3 mutant proteins of the present invention is determined by DNA synthesis by human acute myelogenous leukemia cells (AML). The factor-dependent cell line AML 193 was adapted for use in testing biological activity.

One object of the present invention is to provide hIL-3 muteins and hIL-3 deletion muteins with one or more amino acid substitutions in the polypeptide sequence

which have similar or improved biological activity in relation to native hIL-3 or native (15-125)hIL-3.

The present invention includes mutant polypeptides comprising minimally amino acid residues 15 to 118 of hIL-3 with or without additional amino acid extensions to the N-terminus and/or C-terminus which further contain from one to three or more amino acid substitutions in the amino acid sequence of the polypeptide. It has been found that the (15-125)hIL-3 mutant is more soluble than is hIL-3 when expressed in the cytoplasm of E. coli, and the protein is secreted to the periplasm in E. coli at higher levels compared to native hIL-3.

When expressed in the E. coli cytoplasm, the above-mentioned mutant hIL-3 polypeptides of the present invention may also be constructed with Met-Ala- at the N-terminus so that upon expression the Met is cleaved off leaving Ala at the N-terminus. These mutant hIL-3 polypeptides may also be expressed in E. coli by fusing a signal peptide to the N-terminus. This signal peptide is cleaved from the polypeptide as part of the secretion process. Secretion in E. coli can be used to obtain the correct amino acid at the N-terminus (e.g., Asn^{15} in the (15-125) hIL-3 polypeptide) due to the precise nature of the signal peptidase. This is in contrast to the heterogeneity often observed at the N-terminus of proteins expressed in the cytoplasm in E. coli.

The hIL-3 mutant polypeptides of the present invention may have hIL-3 or hIL-3-like activity. For example, they may possess one or more of the biological activities of native hIL-3 and may be useful in stimulating the production of hematopoietic cells by human or primate progenitor cells. The hIL-3 muteins of the present invention and pharmaceutical compositions containing them may be useful in the treatment of conditions in which hematopoietic cell populations have

been reduced or destroyed due to disease or to treatments such as radiation or chemotherapy.

hIL-3 muteins of the present invention may also be useful as antagonists which block the hIL-3 receptor by binding specifically to it and preventing binding of the agonist.

One potential advantage of the (15-125) hIL-3 muteins of the present invention, particularly those which retain activity similar to or better than that of native hIL-3, is that it may be possible to use a smaller amount of the biologically active mutein to produce the desired therapeutic effect. This may make it possible to reduce the number of treatments necessary to produce the desired therapeutic effect. The use of smaller amounts may also reduce the possibility of any potential antigenic effects or other possible undesirable side effects. For example, if a desired therapeutic effect can be achieved with a smaller amount of polypeptide it may be possible to reduce or eliminate side effects associated with the administration of native IL-3 such as the stimulation of leukotriene and/or histamine release. The hIL-3 muteins of the present invention may also be useful in the activation of stem cells or progenitors which have low receptor numbers. Pharmaceutical compositions containing hIL-3 muteins of the present invention can be administered parenterally, intravenously, or subcutaneously.

In variants which contain an additional cysteine the presence of the cysteine permits the labeling of the protein with ricin which permits targeting ricin and other toxins or tracers using a sulfhydryl linkage to the hIL-3 receptor.

As another aspect of the present invention, there is provided a novel method for producing the novel family of

human IL-3 muteins. The method of the present invention involves culturing a suitable cell or cell line, which has been transformed with a vector containing a DNA sequence coding for expression of a novel hIL-3 mutant polypeptide. Suitable cells or cell lines may be bacterial cells. For example, the various strains of E-coli are well-known as host cells in the field of biotechnology. Examples of such strains include E. coli strains JM101 [Yanish-Perron, et al. (1985)] and MON105 [Obukowicz, et al. (1992)]. Various strains of B subtilis may also be employed in this method. Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention.

Also suitable for use in the present invention are mammalian cells, such as Chinese hamster ovary cells (CHO). General methods for expression of foreign genes in mammalian cells are reviewed in: Kaufman, R. J. (1987) High level production of proteins in mammalian cells, in Genetic Engineering, Principles and Methods, Vol. 9, J. K. Setlow, editor, Plenum Press, New York. An expression vector is constructed in which a strong promoter capable of functioning in mammalian cells drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally fused to the coding region for the hIL-3 variant. For example, plasmids such as pcDNA I/Neo, pRc/RSV, and pRc/CMV (obtained from Invitrogen Corp., San Diego, California) can be used. The eukaryotic secretion signal peptide coding region can be from the hIL-3 gene itself or it can be from another secreted mammalian protein (Bayne, M. L. et al. (1987) Proc. Natl. Acad. Sci. USA 84, 2638-2642). After construction of the vector containing the hIL-3 variant gene, the vector DNA is transfected into mammalian cells. Such cells can be, for example, the COS7, HeLa, BHK, CHO, or mouse L lines. The cells can be cultured, for example, in DMEM media (JRH Scientific). The hIL-3

variant secreted into the media can be recovered by standard biochemical approaches following transient expression 24 - 72 hours after transfection of the cells or after establishment of stable cell lines following selection for neomycin resistance. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al., U.S. Pat. No. 4,419,446. Another suitable mammalian cell line is the monkey COS-1 cell line. A similarly useful mammalian cell line is the CV-1 cell line.

Where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein. In addition, general methods for expression of foreign genes in insect cells using Baculovirus vectors are described in: Summers, M. D. and Smith, G. E. (1987) - A manual of methods for Baculovirus vectors and insect cell culture procedures, Texas Agricultural Experiment Station Bulletin No. 1555. An expression vector is constructed comprising a Baculovirus transfer vector, in which a strong Baculovirus promoter (such as the polyhedron promoter) drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally fused to the coding region for the hIL-3 variant polypeptide. For example, the plasmid pVL1392 (obtained from Invitrogen Corp., San Diego, California) can be used. After construction of the vector carrying the hIL-3 variant gene, two micrograms of this DNA is cotransfected with one microgram of Baculovirus DNA (see Summers & Smith, 1987) into insect cells, strain SF9. Pure recombinant Baculovirus carrying the hIL-3 variant is used to infect cells cultured, for example, in Excell

401 serum-free medium (JRH Biosciences, Lenexa, Kansas). The hIL-3 variant secreted into the medium can be recovered by standard biochemical approaches.

Another aspect of the present invention provides plasmid DNA vectors for use in the method of expression of these novel hIL-3 muteins. These vectors contain the novel DNA sequences described above which code for the novel polypeptides of the invention. Appropriate vectors which can transform microorganisms capable of expressing the hIL-3 muteins include expression vectors comprising nucleotide sequences coding for the hIL-3 muteins joined to transcriptional and translational regulatory sequences which are selected according to the host cells used.

Vectors incorporating modified sequences as described above are included in the present invention and are useful in the production of the hIL-3 mutant polypeptides. The vector employed in the method also contains selected regulatory sequences in operative association with the DNA coding sequences of the invention and capable of directing the replication and expression thereof in selected host cells.

The present invention also includes the construction and expression of (15-125)human interleukin-3 muteins having one or more amino acid substitutions in secretion vectors that optimize accumulation of correctly folded, active polypeptide. While many heterologous proteins have been secreted in E. coli there is still a great deal of unpredictability and limited success (Stader and Silhavy 1990). Full-length hIL-3 is such a protein, where attempts to secrete the protein in E. coli resulted in low levels of secretion. Secretion of the variant (15-125) hIL-3 mutant polypeptides of the present invention as a fusion with a signal peptide such as lamB results in correctly folded protein that can be removed from the periplasm of E. coli by osmotic shock

fractionation. This property of the variant (15-125) hIL-3 muteins allows for the direct and rapid screening for bioactivity of the secreted material in the crude osmotic shock fraction, which is a significant advantage. Furthermore, it provides a means of using the (15-125)hIL-3 muteins to conduct structure activity relationship (SAR) studies of the hIL-3 molecule. A further advantage of secretion of (15-125) hIL-3 muteins fused to the lamB signal peptide is that the secreted polypeptide has the correct N-terminal amino acid (Asn) due to the precise nature of the cleavage of the signal peptide by signal peptidase, as part of the secretion process.

The (15-125)hIL-3 muteins of the present invention may include hIL-3 polypeptides having Met-, Ala- or Met-Ala- attached to the N-terminus. When the muteins are expressed in E. coli, polypeptides with and without Met attached to the N-terminus are obtained. The methionine can in some cases be removed by methionine aminopeptidase.

Amino terminal sequences of some of the hIL-3 muteins made in E. coli were determined using the method described by Hunkapillar et al., (1983). It was found that hIL-3 proteins made in E. coli from genes encoding Met-(15-125)hIL-3 were isolated as Met-(15-125) hIL-3. Proteins produced from genes encoding Met-Ala-(15-125) hIL-3 were produced as Ala-(15-125) hIL-3. The N-termini of proteins made in the cytoplasm of E. coli are affected by posttranslational processing by methionine aminopeptidase (Ben-Bassat et al., 1987) and possibly by other peptidases.

One method of creating the preferred hIL-3 (15-125) mutant genes is cassette mutagenesis [Wells, et al. (1985)] in which a portion of the coding sequence of hIL-3 in a plasmid is replaced with synthetic

oligonucleotides that encode the desired amino acid substitutions in a portion of the gene between two restriction sites. In a similar manner amino acid substitutions could be made in the full-length hIL-3 gene, or genes encoding variants of hIL-3 in which from 1 to 14 amino acids have been deleted from the N-terminus and/or from 1 to 15 amino acids have been deleted from the C-terminus. When properly assembled these oligonucleotides would encode hIL-3 variants with the desired amino acid substitutions and/or deletions from the N-terminus and/or C-terminus. These and other mutations could be created by those skilled in the art by other mutagenesis methods including; oligonucleotide-directed mutagenesis [Zoller and Smith (1982, 1983, 1984), Smith (1985), Kunkel (1985), Taylor, et al. (1985), Deng and Nickoloff (1992)] or polymerase chain reaction (PCR) techniques [Saiki, (1985)].

Pairs of complementary synthetic oligonucleotides encoding portions of the amino terminus of the hIL-3 gene can be made and annealed to each other. Such pairs would have protruding ends compatible with ligation to NcoI at one end. The NcoI site would include the codon for the initiator methionine. At the other end of oligonucleotide pairs, the protruding (or blunt) ends would be compatible with a restriction site that occurs within the coding sequence of the hIL-3 gene. The DNA sequence of the oligonucleotide would encode sequence for amino acids of hIL-3 with the exception of those substituted and/or deleted from the sequence.

The NcoI enzyme and the other restriction enzymes chosen should have recognition sites that occur only once in the DNA of the plasmid chosen. Plasmid DNA can be treated with the chosen restriction endonucleases then ligated to the annealed oligonucleotides. The ligated mixtures can be used to transform competent JM101 cells to resistance to an appropriate antibiotic. Single

colonies can be picked and the plasmid DNA examined by restriction analysis and/or DNA sequencing to identify plasmids with mutant hIL-3 genes.

One example of a restriction enzyme which cleaves within the coding sequence of the hIL-3 gene is ClaI whose recognition site is at codons 20 and 21. The use of ClaI to cleave the sequence of hIL-3 requires that the plasmid DNA be isolated from an E. coli strain that fails to methylate adenines in the DNA at GATC recognition sites. This is because the recognition site for ClaI, ATCGAT, occurs within the sequence GATCGAT which occurs at codons 19, 20 and 21 in the hIL-3 gene. The A in the GATC sequence is methylated in most E. coli host cells. This methylation prevents ClaI from cleaving at that particular sequence. An example of a strain that does not methylate adenines is GM48.

Interpretation of activity of single amino acid mutants in IL-3 (15-125)

As illustrated in Tables 6 and 9, there are certain positions in the IL-3 (15-125) molecule which are intolerant of substitutions, in that most or all substitutions at these positions resulted in a considerable decrease in bioactivity. There are two likely classes of such "down-mutations": mutations that affect overall protein structure, and mutations that interfere directly with the interaction between the IL-3 molecule and its receptor. Mutations affecting the three-dimensional structure of the protein will generally lie in the interior of the protein, while mutations affecting receptor binding will generally lie on the surface of the protein. Although the three-dimensional structure of IL-3 is unknown, there are simple algorithms which can aid in the prediction of the structure. One such algorithm is the use of "helical wheels" (Kaiser,

E.T. & Kezdy, F.J., Science, 223:249-255 (1984)). In this method, the presence of alpha helical protein structures can be predicted by virtue of their amphipathic nature. Helices in globular proteins commonly have an exposed hydrophilic side and a buried hydrophobic side. As a broad generalization, in globular proteins, hydrophobic residues are present in the interior of the protein, and hydrophilic residues are present on the surface. By displaying the amino acid sequence of a protein on such a "helical wheel" it is possible to derive a model for which amino acids in alpha helices are exposed and which are buried in the core of the protein. Such an analysis of the IL-3 (15-125) molecule predicts that the following helical residues are buried in the core:

M19, I20, I23, I24, L27, L58, F61, A64, L68, A71, I74, I77, L78, L81, W104, F107, L111, Y114, L115, L118.

In addition, cysteine residues at positions 16 and 84 are linked by a disulfide bond, which is important for the overall structure or "folding" of the protein. Finally, mutations which result in a major disruption of the protein structure may be expressed at low level in the secretion system used in our study, for a variety of reasons: either because the mis-folded protein is poorly recognized by the secretion machinery of the cell; because mis-folding of the protein results in aggregation, and hence the protein cannot be readily extracted from the cells; or because the mis-folded protein is more susceptible to degradation by cellular proteases. Hence, a block in secretion may indicate which positions in the IL-3 molecule which are important for maintenance of correct protein structure.

In order to retain the activity of a variant of IL-3, it is necessary to retain both the structural integrity of the protein, and retain the specific

residues important for receptor contact. Hence it is possible to define specific amino acid residues in IL-3 (15-125) which must be retained in order to preserve biological activity.

Residues predicted to be important for interaction with the receptor: D21, E22, E43, D44, L48, R54, R94, D103, K110, F113.

Residues predicted to be structurally important: C16, L58, F61, A64, I74, L78, L81, C84, P86, P92, P96, F107, L111, L115, L118.

The hIL-3 muteins of the present invention may be useful in the treatment of diseases characterized by a decreased levels of either myeloid, erythroid, lymphoid, or megakaryocyte cells of the hematopoietic system or combinations thereof. In addition, they may be used to activate mature myeloid and/or lymphoid cells. Among conditions susceptible to treatment with the polypeptides of the present invention is leukopenia, a reduction in the number of circulating leukocytes (white cells) in the peripheral blood. Leukopenia may be induced by exposure to certain viruses or to radiation. It is often a side effect of various forms of cancer therapy, e.g., exposure to chemotherapeutic drugs and of infection or hemorrhage. Therapeutic treatment of leukopenia with these hIL-3 mutant polypeptides of the present invention may avoid undesirable side effects caused by treatment with presently available drugs.

The hIL-3 muteins of the present invention may be useful in the treatment of neutropenia and, for example, in the treatment of such conditions as aplastic anemia, cyclic neutropenia, idiopathic neutropenia, Chdiak-Higashi syndrome, systemic lupus erythematosus (SLE), leukemia, myelodysplastic syndrome and myelofibrosis.

Many drugs may cause bone marrow suppression or hematopoietic deficiencies. Examples of such drugs are AZT, DDI, alkylating agents and anti-metabolites used in chemotherapy, antibiotics such as chloramphenicol, penicillin and sulfa drugs, phenothiazones, tranquilizers such as meprobamate, and diuretics. The hIL-3 muteins of the present invention may be useful in preventing or treating the bone marrow suppression or hematopoietic deficiencies which often occur in patients treated with these drugs.

Hematopoietic deficiencies may also occur as a result of viral, microbial or parasitic infections and as a result of treatment for renal disease or renal failure, e.g., dialysis. The hIL-3 muteins of the present invention may be useful in treating such hematopoietic deficiency.

The treatment of hematopoietic deficiency may include administration of the hIL-3 mutein of a pharmaceutical composition containing the hIL-3 mutein to a patient. The hIL-3 muteins of the present invention may also be useful for the activation and amplification of hematopoietic precursor cells by treating these cells in vitro with the muteins of the present invention prior to injecting the cells into a patient.

Various immunodeficiencies e.g., in T and/or B lymphocytes, or immune disorders, e.g., rheumatoid arthritis, may also be beneficially affected by treatment with the hIL-3 mutant polypeptides of the present invention. Immunodeficiencies may be the result of viral infections e.g. HTLVI, HTLVII, HTLVIII, severe exposure to radiation, cancer therapy or the result of other medical treatment. The hIL-3 mutant polypeptides of the present invention may also be employed, alone or in combination with other hematopoietins, in the treatment

of other blood cell deficiencies, including thrombocytopenia (platelet deficiency), or anemia. Other uses for these novel polypeptides are in the treatment of patients recovering from bone marrow transplants in vivo and ex vivo, and in the development of monoclonal and polyclonal antibodies generated by standard methods for diagnostic or therapeutic use.

Other aspects of the present invention are methods and therapeutic compositions for treating the conditions referred to above. Such compositions comprise a therapeutically effective amount of one or more of the hIL-3 muteins of the present invention in a mixture with a pharmaceutically acceptable carrier. This composition can be administered either parenterally, intravenously or subcutaneously. When administered, the therapeutic composition for use in this invention is preferably in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such a parenterally acceptable protein solution, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

The dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician considering various factors which modify the action of drugs, e.g. the condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. Generally, a daily regimen may be in the range of 0.2 - 150 μg/kg of non-glycosylated IL-3 protein per kilogram of body weight. This dosage regimen is referenced to a standard level of biological activity which recognizes that native IL-3 generally possesses an EC_{50} at or about 10 picoMolar to 100 picoMolar in the AML proliferation assay described herein. Therefore, dosages would be adjusted relative to the activity of a given mutein vs. the activity of native (reference) IL-3 and it

would not be unreasonable to note that dosage regimens may include doses as low as 0.1 microgram and as high as 1 milligram per kilogram of body weight per day. In addition, there may exist specific circumstances where dosages of IL-3 mutein would be adjusted higher or lower than the range of 10 - 200 micrograms per kilogram of body weight. These include co-administration with other CSF or growth factors; co-administration with chemotherapeutic drugs and/or radiation; the use of glycosylated IL-3 mutein; and various patient-related issues mentioned earlier in this section. As indicated above, the therapeutic method and compositions may also include co-administration with other human factors. A non-exclusive list of other appropriate hematopoietins, CSFs and interleukins for simultaneous or serial co-administration with the polypeptides of the present invention includes GM-CSF, CSF-1, G-CSF, Meg-CSF, M-CSF, erythropoietin (EPO), IL-1, IL-4, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, LIF, B-cell growth factor, B-cell differentiation factor and eosinophil differentiation factor, stem cell factor (SCF) also known as steel factor or c-kit ligand, or combinations thereof. The dosage recited above would be adjusted to compensate for such additional components in the therapeutic composition. Progress of the treated patient can be monitored by periodic assessment of the hematological profile, e.g., differential cell count and the like.

Materials and methods for hIL-3 Mutein Expression in E. coli

Unless noted otherwise, all specialty chemicals were obtained from Sigma Co., (St. Louis, MO). Restriction endonucleases, T4 poly-nucleotides kinase, E. coli DNA polymerase I large fragment (Klenow) and T4 DNA ligase were obtained from New England Biolabs (Beverly, Massachusetts) or Boehringer Mannheim (Indianapolis, Indiana). All chemicals and enzymes were used according

to manufacturer's directions.

Escherichia coli strains

Strain JM101: delta (pro lac), supE, thi, F'(traD36, proAB, lacI-Q, lacZdeltaM15) (Messing, 1979). This strain can be obtained from the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, accession number 33876. MON 105 (W3110 rpoH358) (Obukowicz, et al., 1992) is a derivative of W3110 (Bachmann, 1972) and has been assigned ATCC accession number 55204. Strain GM48: dam-3, dcm-6, gal, ara, lac, thr, leu, tonA, tsx (Marinus, 1973) was used to make plasmid DNA that is not methylated at the sequence GATC.

Genes and plasmids

The gene used for hIL-3 production in E. coli was obtained from British Biotechnology Incorporated, Cambridge, England, catalogue number BBG14. This gene is carried on a pUC based plasmid designated pP0518. The human IL-3 gene sequence is from Yang, et al. (1986).

The plasmids used for production of hIL-3 in E. coli contain genetic elements whose use has been described (Olins et al., 1988; Olins and Rangwala, 1990). The replicon used is that of pBR327 [(Bolivar et al. (1977); Soberon et al., 1980] which is maintained at a copy number of about 50 in the cell (Covarrubias, et al., (1981)). A gene encoding the beta-lactamase protein is present on the plasmids. This protein confers ampicillin resistance on the cell. This resistance serves as a selectable phenotype for the presence of the plasmid in the cell.

Intracellular expression plasmids: For cytoplasmic (intracellular) expression vectors the transcription

promoter was derived from the recA gene of E. coli (Sancar et al., 1980). This promoter, designated precA, is contained on 72 base pairs (bp) BglII, BamHI fragment which includes the RNA polymerase binding site and the lexA repressor binding site (the operator). This segment of DNA provides high level transcription that is regulated even when the recA promoter is on a plasmid with the pBR327 origin of replication (Olins et al., 1988) incorporated herein by reference.

Secretion expression plasmids: In secretion expression plasmids the transcription promoter was derived from the *ara B, A,* and *D* genes of *E. coli* (Greenfield *et al.*, 1978). This promoter is designated p*Ara*BAD and is contained on a 323 base pair *SacII, BglII* restriction fragment. The *lam*B secretion leader (Wong *et al.*, 1988, Clement *et al.*, 1981) was fused to the N-terminus of the hIL-3 gene at the recognition sequence for the enzyme *NcoI* (5'CCATGG3'). The hIL-3 genes used were engineered to have a *HindIII* recognition site (5'AAGCTT3') following the coding sequence of the gene. Downstream of the gene is a 550 bp fragment containing the origin of replication of the single stranded phage f1 [Olins and Rangwala (1989)].

These hIL-3 variants were expressed as a fusion with the lamB signal peptide operatively joined to the araBAD promoter (Greenfield, 1978) and the g10-L ribosome binding site (Olins et al. 1988). The signal peptide is removed as part of the secretion process. The processed form was selectively released from the periplasm by osmotic shock as a correctly folded and fully active molecule. Secretion of (15-125) hIL-3 was further optimized by using low inducer (arabinose) concentration and by growth at 30°C. These conditions resulted in lower accumulation levels of unprocessed lamB signal peptide (15-125) hIL-3 fusion, maximal accumulation levels of processed (15-125) hIL-3 and selective release

of (15-125) hIL-3 by osmotic shock fractionation. The use of a tightly regulated promoter such as araBAD from which the transcription level and hence the expression level can be modulated allowed for the optimization of secretion of (15-125) hIL-3.

The ribosome binding site (RBS) used is that from gene 10 of phage T7 (Olins et al., 1988). This is encoded in a 100 base pair (bp) fragment placed adjacent to precA. In the plasmids used herein, the recognition sequence for the enzyme NcoI (5'CCATGG3') follows the g10-L RBS. It is at this NcoI site that the hIL-3 genes are joined to the plasmid. It is expected that the nucleotide sequence at this junction will be recognized in mRNA as a functional start site for translation (Olins et al., 1988). The hIL-3 genes used were engineered to have a HindIII recognition site (5'AAGCTT3') following the coding sequence of the gene. Downstream of the gene is a 550 base pair fragment containing the origin of replication of the single stranded phage f1 (Dente et al., 1983; Olins, et al., 1990) both incorporated herein by reference. A plasmid containing these elements is pMON2341. Another plasmid containing these elements is pMON5847 which has been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 under the accession number ATCC 68912.

Synthesis of Oligonucleotides

Oligonucleotides were synthesized by the cyanoethyl method (Adams et al. 1983, McBride, et al. 1983, Sinba et al., 1984) on Nucleotide Synthesizer model 380A or 380B from Applied Biosystems, Inc. (Foster City, California). Some oligonucleotides were purchased from Genosys Biotechnologies Inc. (The Woodlands, Texas) or Midland Certified Reagent Co. (Midland, Texas). The degenerate oligonucleotides were synthesized by machine mixing an equal molar ratio of the desired nucleosides in the

condensation reaction at degenerate positions. Oligonucleotides were purified by polyacrylamide gel electrophoresis at concentrations from 12 - 20% (19:1 crosslinked) in 0.5X Tris borate (TBE) buffer (0.045 M Tris, 0.045 M boric acid, 1.25 mM EDTA) as described by Atkinson (1984). The oligonucleotides were desalted by passage through a Nensorb 20 column obtained from DuPont/New England Nuclear (Boston, Massachusetts) using a PREP Automated Sample Processor obtained from DuPont, Co. (Wilmington, Delaware).

Quantitation of synthetic oligonucleotides

Synthetic oligonucleotides were resuspended in water (100 µl) and quantitated by reading the absorbance at 260nm on a Beckman DU40 Spectrophotometer (Irvine, California) using a one centimeter by one millimeter quartz cuvette (Maniatis, 1982). The concentration was determined using an extinction coefficient of 1×10^4 (Voet et al., 1963; Mahler and Cordes, 1966). The oligonucleotide was then diluted to the desired concentration.

Quantitation of synthetic DNA fragments can also be achieved by adding 10 to 100 picomoles of DNA to a solution containing kinase buffer (25 mM Tris pH 8.0, 10 mM $MgCl_2$, 10 mM DTT and 2 mM spermidine). To the reaction mix is added ATP to 20 micromolar, ATP radiolabeled at the gamma phosphate (5000-10,0000 dpm/pmol) and 5 units of T4 polynucleotide kinase. Radiolabelled material is obtained from New England Nuclear (Boston, Massachusetts). The 10 microliter mixture is incubated at 37°C for one hour. A 1 microliter aliquot of the mixture is chromatographed on DEAE paper (DE81 from Whatman) in 0.35 M ammonium bicarbonate. The counts that remain at the origin are used to determine the concentration of the synthetic DNA.

Recombinant DNA methods

Isolation of plasmid DNA from E. coli cultures was performed as described (Birnboim and Doly, 1979). Some DNAs were purified by Magic™ miniprep columns, available from Promega (Madison, Wisconsin).

Purified plasmid DNA was treated with restriction endonucleases according to manufacturer's instructions. Analysis of the DNA fragments produced by treatment with restriction enzymes was done by agarose or polyacrylamide gel electrophoresis. Agarose (DNA grade from Fisher, Pittsburgh PA.) was used at a concentration of 1.0% in a Tris-acetate running buffer (0.04 M Tris-acetate, 0.001M EDTA). Polyacrylamide (BioRad, Richmond CA.) was used at a concentration of 6% (19:1 crosslinked) in 0.5 X Tris-borate buffer (0.045 M Tris, 0.045 M boric acid, 1.25 mM EDTA), hereafter referred to as PAGE.

DNA polymerase I, large fragment, Klenow enzyme was used according to manufacturer's instructions to catalyze the addition of mononucleotides from 5' to 3' of DNA fragments which had been treated with restriction enzymes that leave protruding ends. The reactions were incubated at 65°C for 10 minutes to heat inactivate the Klenow enzyme.

The synthetic oligonucleotides were made without 5' or 3' terminal phosphates. In cases where such oligonucleotides were ligated end to end, the oligonucleotides were treated at a concentration of 10 picomoles per microliter with T4 polynucleotide kinase in the following buffer: 25 mM Tris, pH 8.0, 10 mM $MgCl_2$, 10 mM dithiothreitol, 2 mM spermidine, 1 mM rATP. After incubation for 30 minutes at 37°C, the samples were incubated at 65°C for five minutes to heat inactivate the kinase.

Synthetic gene assembly

The (15-125) hIL-3 gene was divided into four regions separated by five convenient restriction sites. In each of the four regions synthetic oligonucleotides were designed so that they would anneal in complementary pairs, with protruding single stranded ends "or blunt ends" and when the pairs were properly assembled would result in a DNA sequence that encoded a portion of the hIL-3 gene. Amino acid substitutions in the hIL-3 gene were made by designing the oligonucleotides to encode the desired substitutions. The complementary oligonucleotides were annealed at concentration of 1 picomole per microliter in ligation buffer plus 50mM NaCl. The samples were heated in a 100 ml beaker of boiling water and permitted to cool slowly to room temperature. One picomole of each of the annealed pairs of oligonucleotides were ligated with approximately 0.2 picomoles of plasmid DNA, digested with the appropriate restriction enzymes, in ligation buffer (25 mM Tris pH 8.0, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, 2mM spermidine) with T4 DNA ligase obtained from New England Biolabs (Beverly, Massachusetts) in a total volume of 20 µl at room temperature overnight.

DNA fragments were isolated from agarose gels by intercepting the restriction fragments on DEAE membranes from Schleicher and Schuell (Keene, New Hampshire) and eluting the DNA in 10 mM Tris, 1 mM EDTA, 1 M NaCl at 55°C for 1 hour, according to manufacturer's directions. The solutions containing the DNA fragment were concentrated and desalted by using Centricon 30 concentrators from Amicon (W.R. Grace, Beverly MA) according to the manufacturer's directions. Ligations were performed at 15°C overnight, except as noted, in ligation buffer (66 mM Tris pH 7.5, 6.6 mM $MgCl_2$, 1 mM dithiothreitol, 0.4 mM ATP) with T_4 ligase obtained from New England Biolabs (Beverly, Massachusetts).

Polymerase Chain Reaction

Polymerase Chain Reaction (hereafter referred to as PCR) techniques (Saiki, 1985) used the reagent kit and thermal cycler from Perkin-Elmer Cetus (Norwalk, CT.). PCR is based on a thermostable DNA polymerase from *Thermus aquaticus*. The PCR technique is a DNA amplification method that mimics the natural DNA replication process in that the number of DNA molecules doubles after each cycle, in a way similar to in vivo replication. The DNA polymerase mediated extension is in a 5′→3′ direction. The term "primer" as used herein refers to an oligonucleotide sequence that provides an end to which the DNA polymerase can add nucleotides that are complementary to a nucleotide sequence. The latter nucleotide sequence is referred to as the "template", to which the primers are annealed. The amplified PCR product is defined as the region comprised between the 5′ ends of the extension primers. Since the primers have defined sequences, the product will have discrete ends, corresponding to the primer sequences. The primer extension reaction was carried out using 20 picomoles (pmoles) of each of the oligonucleotides and 1 picogram of template plasmid DNA for 35 cycles (1 cycle is defined as 94°C for one minute, 50°C for two minutes and 72°C for three minutes). The reaction mixture was extracted with an equal volume of phenol/chloroform (50% phenol and 50% chloroform, volume to volume) to remove proteins. The aqueous phase, containing the amplified DNA, and solvent phase were separated by centrifugation for 5 minutes in a microcentrifuge (Model 5414 Eppendorf Inc, Fremont CA). To precipitate the amplified DNA the aqueous phase was removed and transferred to a fresh tube to which was added 1/10 volume of 3M NaOAc (pH 5.2) and 2.5 volumes of ethanol (100% stored at minus 20°C). The solution was mixed and placed on dry ice for 20 minutes. The DNA was pelleted by centrifugation for 10 minutes in a

microcentrifuge and the solution was removed from the pellet. The DNA pellet was washed with 70% ethanol, ethanol removed and dried in a speedvac concentrator (Savant, Farmingdale, New York). The pellet was resuspended in 25 microliters of TE (20mM Tris-HCl pH 7.9, 1mM EDTA). Alternatively the DNA was precipitated by adding equal volume of 4M NH_4OAc and one volume of isopropanol [Treco, (1989)]. The solution was mixed and incubated at room temperature for 10 minutes and centrifuged. These conditions selectively precipitate DNA fragments larger than ~ 20 bases and were used to remove oligonucleotide primers. One quarter of the reaction was digested with restriction enzymes [Higuchi, (1989)] and on completion heated to 70°C to inactivate the enzymes.

Two step site-directed PCR mutagenesis

Single amino acid substitution variants were created at positions 17-123 of hIL-3 in two site-directed mutagenesis steps by PCR (Bauer *et al.* manuscript in preparation).

The single amino acid substitution variants at positions 94-105 of hIL-3 were created as described below. In the first mutagenesis step plasmid DNA, containing the hIL-3 gene (amino acids 15-125), was the template in the PCR reaction. The DNA sequence of one of the oligonucleotide primers was designed to replace 12 base in the hIL-3 gene (15-125) with 12 bases encoding two translation stop codons (5'TAATAA3'), followed by the recognition sequence (5'GTCGAC3') restriction enzyme *SalI*. This 12 base sequence was substituted in the hIL-3 gene following the codon for amino acids 93, 97 and 101. Plasmids containing these mutagenized genes served as the templates for the second mutagenesis step.

In the second mutagenesis step, the 12 base

substitution introduced in the first mutagenesis step, was replaced using a 32 fold degenerate oligonucleotide. The degenerate oligonucleotides were synthesized by machine mixing an equal molar ratio of the desired nucleosides in the condensation reaction at degenerate positions. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon. The other bases in the oligonucleotides corresponded to the hIL-3 sequence. The degenerate oligonucleotides theoretically contain 32 different codons, encoding all 20 amino acids and one translation stop codon, at a single position. At the other 9 bases the DNA sequence was restored to encode the native hIL-3 protein sequence. This pool of single amino acid substitutions at a single position is referred to as a "library". This two step PCR site-directed mutagenesis approach was used to facilitate the identification of single amino acid substitution variants by differential DNA hybridization.

The single amino acid substitution variants at positions 17-93 and 106-123 of hIL-3 (15-125) were created as described below. In the first mutagenesis step plasmid DNA, containing the hIL-3 gene (15-125), was the template in the PCR reaction. The DNA sequence of one of the oligonucleotide primers was designed to delete 18 bases in the hIL-3 gene that encode the following amino acids; 17-22, 23-28, 29-34, 35-40, 41-46, 47-52, 53-58, 59-64, 65-70, 71-76, 77-82, 83-88, 88-93, 106-111, 112-117 and 118-123. Plasmids containing these deletion genes served as the templates for the second mutagenesis step.

In the second mutagenesis step the 18 base deletion, created in the first mutagenesis step, was restored using a 32 fold degenerate oligonucleotide. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a

single codon. The other bases in the oligonucleotides corresponded to the hIL-3 sequence. The degenerate oligonucleotides theoretically contain 32 different codons, encoding all 20 amino acids and one translation stop codon, at a single position. At the other 9 bases the DNA sequence was restored to encode the native hIL-3 protein sequence. This pool of single amino acid substitutions at a single position is referred to as a "library". This two step PCR site-directed mutagenesis approach was used to facilitate the identification of single amino acid substitution variants by differential DNA hybridization.

Recovery of recombinant plasmids from ligation mixes and transformation of E. coli cells with recombinant plasmid DNA

E. coli JM101 cells were made competent to take up DNA. Typically, 20 to 100 ml of cells were grown in LB medium to a density of approximately 150 Klett units and then collected by centrifugation. The cells were resuspended in one half culture volume of 50 mM $CaCl_2$ and held at 4°C for one hour. The cells were again collected by centrifugation and resuspended in one tenth culture volume of 50 mM $CaCl_2$. DNA was added to a 150 microliter volume of these cells, and the samples were held at 4°C for 30 minutes. The samples were shifted to 42°C for one minute, one milliliter of LB was added, and the samples were shaken at 37°C for one hour. Cells from these samples were spread on plates containing ampicillin to select for transformants. The plates were incubated overnight at 37°C. Single colonies were picked and grown in LB supplemented with ampicillin overnight at 37°C with shaking. From these cultures DNA was isolated for restriction analysis.

Typically plasmids were constructed, using methods described herein or by references cited herein, as

follows except as noted in examples included herein. DNA fragments were purified from agarose or polyacrylamide gels. Purified DNA fragments were ligated and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin containing plates. Plasmid DNA was isolated from a single colony grown in LB Broth and screened by restriction analysis for the desired construct and sequenced to determine that the DNA sequence was correct.

Culture media

LB medium (Maniatis et al., 1982) was used for growth of cells for DNA isolation. M9 minimal medium supplemented with 1.0% casamino acids, acid hydrolyzed casein, Difco (Detroit, Michigan) was used for cultures in which recombinant hIL-3 was produced. The ingredients in the M9 medium were as follows: 3g/liter KH_2PO_4, 6g/l Na_2HPO_4, 0.5 g/l NaCl, 1 g/l NH_4Cl, 1.2 mM $MgSO_4$, 0.025 mM $CaCl_2$, 0.2% glucose (0.2% glycerol with the AraBAD promoter), 1% casamino acids, 0.1 ml/l trace minerals (per liter 108 g $FeCl_3 \cdot 6H_2O$, 4.0 g $ZnSO_4 \cdot 7H_2O$, 7.0 $CoCl_2 \cdot 2H_2O$, 7.0 g $Na_2MoO_4 \cdot 2H_2O$, 8.0 g $CuSO_4 \cdot 5H_2O$, 2.0 g H_3BO_3, 5.0 g $MnSO_4 \cdot H_2O$, 100 ml concentrated HCl). Bacto agar from Difco was used for solid media and ampicillin (Polycillin-N from Bristol-Meyers, Evansville, Indiana) was added to both liquid and solid LB media at 200 micrograms per milliliter.

DNA sequence analysis

The nucleotide sequencing of plasmid DNA was performed using a Genesis 2000 sequencer obtained from DuPont (Wilmington, Delaware) according to the methods of Prober et al. (1987) and Sanger et al. (1977). Some DNA sequences were determined using Sequenase™ polymerase according to the protocol of its supplier, U.S. Biochemicals (Cleveland, Ohio).

Production of recombinant hIL-3 muteins in E. coli with vectors employing the recA promoter

E. coli strains harboring the plasmids of interest were grown at 37°C in M9 plus casamino acids medium with shaking in a Gyrotory water bath Model G76 from New Brunswick Scientific (Edison, New Jersey). Growth was monitored with a Klett Summerson meter (green 54 filter), Klett Mfg. Co. (New York, New York). At a Klett value of approximately 150, an aliquot of the culture (usually one milliliter) was removed for protein analysis. To the remaining culture, nalidixic acid (10mg/ml) in 0.1 N NaOH was added to a final concentration of 50 µg/ml. The cultures were shaken at 37°C for three to four hours after addition of nalidixic acid. A high degree of aeration was maintained throughout the bacterial growth in order to achieve maximal production of the desired gene product. The cells were examined under a light microscope for the presence of refractile bodies (RBs). One milliliter aliquots of the culture were removed for analysis of protein content.

Production of recombinant hIL-3 proteins from the *AraBAD* promoter in *E. coli*

E. coli strains harboring the plasmids of interest were grown at 30°C with shaking in M9 medium plus casamino acids and glycerol. Growth was monitored with a Klett Summerson colorimeter, using a green 54 filter. At a Klett value of about 150, an aliquot of the culture (usually one milliliter) was removed for protein analysis. To the remaining culture, 20% arabinose was added to a final concentration of 0.05%. The cultures were shaken at 30°C for three to four hours after addition of arabinose. A high degree of aeration was maintained throughout the bacterial growth in order to achieve maximal production of the desired gene product.

One milliliter aliquots of the culture were removed for analysis of protein content.

Secretion and osmotic shock

Three hour post induction samples were fractionated by osmotic shock [Neu and Heppel (1965)]. The Klett value of the cultures was determined and 1 ml of cells were centrifuged in a Sigma microcentrifuge (West Germany) model 202MK in 1.5 mls snap top microcentrifuge tubes for 5 minutes at 10,000 rpm. The cell pellet was resuspended very gently by pipeting in a room temperature sucrose solution (20% sucrose w/v, 30mM Tris-Hcl pH7.5, 1mM EDTA), using 1µl/1 Klett unit. Following a 10 minute incubation at room temperature, the cells were centrifuged for 5 minutes at 10,000 rpm. The sucrose fraction was carefully removed from the cell pellet. The cell pellet was then resuspended very gently by pipeting in ice cold distilled water, using 1µl/1 Klett unit. Following a 10 minute incubation on ice, the cells were centrifuged for 5 minutes at 12,000 rpm. The water fraction was carefully removed. Equal volumes of the sucrose and water fractions were pooled and aliquoted to provide samples for ELISA and biological activity screening.

Analysis of protein content of E. coli cultures producing hIL-3 mutant polypeptides

Bacterial cells from cultures treated as described above were collected from the medium by centrifugation. Aliquots of these cells were resuspended in SDS loading buffer (4X: 6 g SDS, 10 ml beta-mercaptoethanol, 25 ml upper Tris gel stock (0.5 M Tris HCl pH 6.8, 0.4% SDS) brought to 50 ml with glycerol, 0.2% bromophenol blue was added) at a concentration of one microliter per Klett unit. These samples were incubated at 85°C for five minutes and vortexed. Five or ten microliter aliquots of

these samples were loaded on 15% polyacrylamide gels prepared according to the method of Laemmli (1970). Protein bands were visualized by staining the gels with a solution of acetic acid, methanol and water at 5:1:5 (volume to volume) ratio to which Coomassie blue had been added to a final concentration of 1%. After staining, the gels were washed in the same solution without the Coomassie blue and then washed with a solution of 7% acetic acid, 5% methanol. Gels were dried on a gel drier Model SE1160 obtained from Hoeffer (San Francisco, California). The amount of stained protein was measured using a densitometer obtained from Joyce-Loebl (Gateshead, England). The values obtained were a measure of the amount of the stained hIL-3 protein compared to the total of the stained protein of the bacterial cells.

Western blot analysis of hIL-3 muteins made in E. coli

In some E. coli cultures producing hIL-3, the level of accumulation of the hIL-3 protein is lower than 5% of total bacterial protein. To detect hIL-3 produced at this level, Western blot analysis was used. Proteins from cultures induced with nalidixic acid or arabinose were run on polyacrylamide gels as described above except that volumes of sample loaded were adjusted to produce appropriate signals. After electrophoresis, the proteins were electroblotted to APT paper, Transa-bind, Schleicher and Schuell (Keene, New Hampshire) according to the method of Renart et al. (1979). Antisera used to probe these blots had been raised in rabbits, using peptides of the sequence of amino acids 20 to 41 and 94 to 118 of hIL-3 as the immunogens. The presence of bound antibody was detected with Staphylococcal protein A radiolabeled with ^{125}I, obtained from New England Nuclear (Boston, Massachusetts).

Fractionation of E. coli cells producing hIL-3 proteins in the cytoplasm

Cells from E. coli cultures harboring plasmids that produce hIL-3 muteins were induced with nalidixic acid. After three hours, the hIL-3 muteins accumulated in refractile bodies. The first step in purification of the hIL-3 muteins was to sonicate cells. Aliquots of the culture were resuspended from cell pellets in sonication buffer: 10 mM Tris, pH 8.0, 1 mM EDTA, 50 mM NaCl and 0.1 mM PMSF. These resuspended cells were subjected to several repeated sonication bursts using the microtip from a Sonicator cell disrupter, Model W-375 obtained from Heat Systems-Ultrasonics Inc. (Farmingdale, New York). The extent of sonication was monitored by examining the homogenates under a light microscope. When nearly all of the cells had been broken, the homogenates were fractionated by centrifugation. The pellets, which contain most of the refractile bodies, are highly enriched for hIL-3 muteins.

Methods: Extraction, Refolding and Purification of Interleukin-3 (IL-3) Muteins Expressed as Refractile Bodies in E. coli.

Extraction of refractile bodies (RB's):

For each gram of RB's (and typically one gram is obtained from a 300 ml E. coli culture), 5 ml of a solution containing 6M guanidine hydrochloride (GnHCl), 50 mM 2-N-cyclohexylaminoethanesulfonic acid (CHES) pH 9.5 and 20 mM dithiothreitol (DTT) was added. The RB's were extracted with a Bio-Homogenizer for 15-30 seconds and gently rocked for 2 hours at 5 degrees centigrade (5°C) to allow the protein to completely reduce and denature.

Refolding of the IL-3 muteins

The protein solution was transferred to dialysis tubing (1000 molecular weight cut-off) and dialyzed

against at least 100 volumes of 4M GnHCl - 50 mM CHES pH 8.0. The dialysis was continued overnight at 5°C while gently stirring. Subsequently dialysis was continued against at least 100 volumes of 2M GnHCl - 50 mM CHES pH 8.0 and dialyzed overnight at 5°C while gently stirring.

Purification of the IL-3 muteins

The protein solution was removed from the dialysis tubing and acidified by the addition of 40% acetonitrile (CH_3CN) - 0.2% trifluoroacetic acid (TFA) to a final concentration of 20% CH_3CN - 0.1% TFA. This was centrifuged (16,000 x g for 5 minutes) to clarify and the supernatant was loaded onto a Vydac C-18 reversed phase column (10x250 mm) available from Vydac (Hesperia, California) previously equilibrated in 20% CH_3CN - 0.1% TFA. The column was eluted with a linear gradient (0.2% CH_3CN/minute) between 40 - 50% CH_3CN - 0.1% TFA at a flow rate of 3 ml/minute while collecting 1.5 ml fractions. The fractions were analyzed by polyacrylamide gel electrophoresis (SDS-PAGE) and the appropriate fractions pooled. The pooled material was dried by lyophilization or in a Speed Vac concentrator. The dry powder was reconstituted with 10 mM ammonium bicarbonate pH 7.5, centrifuged (16,000 x g for 5 minutes) to clarify and assayed for protein concentration by the method of Bradford (1976) with bovine serum albumin as the standard. Such protein can be further analyzed by additional techniques such as, SDS-PAGE, electrospray mass spectrometry, reverse phase HPLC, capillary zone electrophoresis, amino acid composition analysis, and ELISA (enzyme-linked immunosorbent assay).

hIL-3 SANDWICH ELISA

IL-3 protein concentrations were determined using a sandwich ELISA based on an affinity purified polyclonal

goat anti-rhIL-3. Microtiter plates (Dynatech Immulon II) were coated with 150 µl goat-anti-rhIL-3 at a concentration of approximately 1 µg/ml in 100 mM NaHCO3, pH 8.2. Plates were incubated overnight at room temperature in a chamber maintaining 100% humidity. Wells were emptied and the remaining reactive sites on the plate were blocked with 200 µl of solution containing 10 mM PBS, 3% BSA and 0.05% Tween 20, pH 7.4 for 1 hour at 37° C and 100% humidity. Wells were emptied and washed 4X with 150 mM NaCl containing 0.05% Tween 20 (wash buffer). Each well then received 150 µl of dilution buffer (10 mM PBS containing 0.1% BSA, 0.01% Tween 20, pH 7.4), containing rhIL-3 standard, control, sample or dilution buffer alone. A standard curve was prepared with concentrations ranging from 0.125 ng/ml to 5 ng/ml using a stock solution of rhIL-3 (concentration determined by amino acid composition analysis). Plates were incubated 2.5 hours at 37° C and 100% humidity. Wells were emptied and each plate was washed 4X with wash buffer. Each well then received 150 µl of an optimal dilution (as determined in a checkerboard assay format) of goat anti-rhIL-3 conjugated to horseradish peroxidase. Plates were incubated 1.5 hours at 37° C and 100% humidity. Wells were emptied and each plate was washed 4X with wash buffer. Each well then received 150 ul of ABTS substrate solution (Kirkegaard and Perry). Plates were incubated at room temperature until the color of the standard wells containing 5 ng/ml rhIL-3 had developed enough to yield an absorbance between 0.5-1.0 when read at a test wavelength of 410 nm and a reference wavelength of 570 nm on a Dynatech microtiter plate reader. Concentrations of immunoreactive rhIL-3 in unknown samples were calculated from the standard curve using software supplied with the plate reader.

AML Proliferation Assay for Bioactive Human Interleukin-3

The factor-dependent cell line AML 193 was obtained

from the American Type Culture Collection (ATCC, Rockville, MD). This cell line, established from a patient with acute myelogenous leukemia, is a growth factor dependent cell line which displayed enhanced growth in GM/CSF supplemented medium (Lange, B., et al., (1987); Valtieri, M., et al., (1987). The ability of AML 193 cells to proliferate in the presence of human IL-3 has also been documented. (Santoli, D., et al., (1987)). A cell line variant was used, AML 193 1.3, which was adapted for long term growth in IL-3 by washing out the growth factors and starving the cytokine dependent AML 193 cells for growth factors for 24 hours. The cells were then replated at $1x10^5$ cells/well in a 24 well plate in media containing 100 U/ml IL-3. It took approximately 2 months for the cells to grow rapidly in IL-3. These cells were maintained as AML 193 1.3 thereafter by supplementing tissue culture medium (see below) with human IL-3.

AML 193 1.3 cells were washed 6 times in cold Hanks balanced salt solution (HBSS, Gibco, Grand Island, NY) by centrifuging cell suspensions at 250 x g for 10 minutes followed by decantation of supernatant. Pelleted cells were resuspended in HBSS and the procedure was repeated until six wash cycles were completed. Cells washed six times by this procedure were resuspended in tissue culture medium at a density ranging from 2×10^5 to 5×10^5 viable cells/ml. This medium was prepared by supplementing Iscove's modified Dulbecco's Medium (IMDM, Hazleton, Lenexa, KS) with albumin, transferrin, lipids and 2-mercaptoethanol. Bovine albumin (Boehringer-Mannheim, Indianapolis, IN) was added at 500 µg/ml; human transferrin (Boehringer-Mannheim, Indianapolis, IN) was added at 100 µg/ml; soybean lipid (Boehringer-Mannheim, Indianapolis, IN) was added at 50 µg/ml; and 2-mercaptoethanol (Sigma, St. Louis, MO) was added at 5×10^{-5}M.

Serial dilutions of human interleukin-3 or human interleukin-3 variant protein (hIL-3 mutein) were made in triplicate series in tissue culture medium supplemented as stated above in 96 well Costar 3596 tissue culture plates. Each well contained 50 µl of medium containing interleukin-3 or interleukin-3 variant protein once serial dilutions were completed. Control wells contained tissue culture medium alone (negative control). AML 193 1.3 cell suspensions prepared as above were added to each well by pipetting 50 µl (2.5×10^4 cells) into each well. Tissue culture plates were incubated at 37°C with 5% CO_2 in humidified air for 3 days. On day 3, 0.5 µCi ^{3}H-thymidine (2 Ci/mM, New England Nuclear, Boston, MA) was added in 50 µl of tissue culture medium. Cultures were incubated at 37°C with 5% CO_2 in humidified air for 18-24 hours. Cellular DNA was harvested onto glass filter mats (Pharmacia LKB, Gaithersburg, MD) using a TOMTEC cell harvester (TOMTEC, Orange, CT) which utilized a water wash cycle followed by a 70% ethanol wash cycle. Filter mats were allowed to air dry and then placed into sample bags to which scintillation fluid (Scintiverse II, Fisher Scientific, St. Louis, MO or BetaPlate Scintillation Fluid, Pharmacia LKB, Gaithersburg, MD) was added. Beta emissions of samples from individual tissue culture wells were counted in a LKB Betaplate model 1205 scintillation counter (Pharmacia LKB, Gaithersburg, MD) and data was expressed as counts per minute of ^{3}H-thymidine incorporated into cells from each tissue culture well. Activity of each human interleukin-3 preparation or human interleukin-3 variant preparation was quantitated by measuring cell proliferation (^{3}H-thymidine incorporation) induced by graded concentrations of interleukin-3 or interleukin-3 variant. Typically, concentration ranges from 0.05 pM - 10^5 pM are quantitated in these assays. Activity is determined by measuring the dose of interleukin-3 or interleukin-3 variant which provides 50% of maximal proliferation [EC_{50} = 0.5 x (maximum average counts per minute of ^{3}H-thymidine incorporated per well

among triplicate cultures of all concentrations of interleukin-3 tested - background proliferation measured by ^{3}H-thymidine incorporation observed in triplicate cultures lacking interleukin-3]. This EC_{50} value is also equivalent to 1 unit of bioactivity. Every assay was performed with native interleukin-3 as a reference standard so that relative activity levels could be assigned.

Relative biological activities of some IL-3 muteins of the present invention are shown in Table 1. The Relative Biological Activity of IL-3 mutants is calculated by dividing the EC_{50} of (1-133) hIL-3 by the EC_{50} of the mutant. The Relative Biological Activity may represent the average of replicate assays.

TABLE 1

BIOLOGICAL ACTIVITY OF IL-3 MUTEINS

Plasmid Code	Polypeptide Structure	Relative Biological Activity
Reference	(1-133)hIL-3	1.0
pMON13286	[SEQ ID NO. 69]	8.0
pMON13304	[SEQ ID NO. 66]	3.2

* The Relative Biological Activity of IL-3 mutants is calculated by dividing the EC_{50} of (1-133) hIL-3 by the EC_{50} of the mutant.

The following assay is used to measure IL-3 mediated sulfidoleukotriene release from human mononuclear cells.

IL-3 mediated sulfidoleukotriene release from human mononuclear cells

Heparin-containing human blood was collected and layered onto an equal volume of Ficoll-Paque (Pharmacia # 17-0840-02) ready to use medium (density 1.077 g/ml.). The Ficoll was warmed to room temperature prior to use and clear 50 ml polystyrene tubes were utilized. The Ficoll gradient was spun at 300 x g for 30 minutes at room temperature using a H1000B rotor in a Sorvall RT6000B refrigerated centrifuge. The band containing the mononuclear cells was carefully removed, the volume adjusted to 50 mls with Dulbecco's phosphate-buffered saline (Gibco Laboratories cat. # 310-4040PK), spun at 400 x g for 10 minutes at 4°C and the supernatant was

carefully removed. The cell pellet was washed twice with HA Buffer [20 mM Hepes (Sigma # H-3375), 125 mM NaCl (Fisher # S271-500), 5 mM KCl (Sigma # P-9541), 0.5 mM glucose (Sigma # G-5000),0.025% Human Serum Albumin (Calbiochem # 126654) and spun at 300 x g, 10 min., 4°C. The cells were resuspended in HACM Buffer (HA buffer supplemented with 1 mM CaCl2 (Fisher # C79-500) and 1 mM MgCl2 (Fisher # M-33) at a concentration of 1 x 106 cells/ml and 180 µl were transferred into each well of 96 well tissue culture plates. The cells were allowed to acclimate at 37°C for 15 minutes. The cells were primed by adding 10 µls of a 20 X stock of various concentrations of cytokine to each well (typically 100000, 20000, 4000, 800, 160, 32, 6.4, 1.28, 0 fM IL3). The cells were incubated for 15 minutes at 37°C. Sulfidoleukotriene release was activated by the addition of 10 µls of 20 X (1000 nM) fmet-leu-phe (Calbiochem # 344252) final concentration 50nM FMLP and incubated for 10 minutes at 37°C. The plates were spun at 350 x g at 4°C for 20 minutes. The supernatants were removed and assayed for sulfidoleukotrienes using Cayman's Leukotriene C4 EIA kit (Cat. #420211) according to manufacturers' directions. Native (15-125)hIL-3 was run as a standard control in each assay.

Native hIL-3 possesses considerable inflammatory activity and has been shown to stimulate synthesis of the arachidonic acid metabolites LTC_4, LTD_4, and LTE_4; histamine synthesis and histamine release. Human clinical trials with native hIL-3 have documented inflammatory responses (Biesma, et al., BLOOD, 80:1141-1148 (1992) and Postmus, et al., J. CLIN. ONCOL., 10:1131-1140 (1992)). A recent study indicates that leukotrienes are involved in IL-3 actions in vivo and may contribute significantly to the biological effects of IL-3 treatment (Denzlinger, C., et al., BLOOD, 81:2466-2470 (1993))

Some muteins of the present invention may have an improved therapeutic profile as compared to native hIL-3 or (15-125)hIL-3. For example, some muteins of the present invention may have a similar or more potent growth factor activity relative to native hIL-3 or (15-125)hIL-3 without having a similar or corresponding increase in the stimulation of leukotriene or histamine. These muteins would be expected to have a more favorable therapeutic profile since the amount of polypeptide which needs to be given to achieve the desired growth factor activity (e. g. cell proliferation) would have a lesser leukotriene or histamine stimulating effect. In studies with native hIL-3, the stimulation of inflammatory factors has been an undesirable side effect of the treatment. Reduction or elimination of the stimulation of mediators of inflammation would provide an advantage over the use of native hIL-3.

Some muteins of the present invention may have antigenic profiles which differ from that of native hIL-3. For example, in a competition ELISA with an affinity purified polyclonal goat anti-hIL-3 antibody, native hIL-3 significantly blocked the binding of labeled hIL-3 to polyclonal anti-hIL-3 antibody. Some polypeptides of the present invention, particularly those with several amino acids differing from those of native hIL-3, fail to block the binding of hIL-3 to anti-hIL-3 antibody.

Table 2 lists the sequences of some oligonucleotides used in making the muteins of the present invention.

Table 3 lists the amino acid sequence of native (15-125)hIL-3 (Peptide #1) and the amino acid sequences of some mutant polypeptides of the present invention. The sequences are shown with the amino acid numbering corresponding to that of native hIL-3 [FIG. 1].

TABLE 2

OLIGONUCLEOTIDES

Oligo #1

AATTCCGTCG TAAACTGACC TTCTATCTGA AAACCTTGGA GAACGCGCAG GCTCAACAGT AATA [SEQ ID NO: 8]

Oligo #2

AGCTTATTAC TGTTGAGCCT GCGCGTTCTC CAAGGTTTTC AGATAGAAGG TCAGTTTACG ACGG [SEQ ID NO: 9]

Oligo #3

CTAGCCACGG CCGCACCCAC GCGACATCCA ATCCATATCA AGGACGGTGA CTGGAATG [SEQ ID NO:24]

Oligo #4

TTAACATTCC AGTCACCGTC CTTGATATGG ATTGGATGTC GCGTGGGTGC GGCCGTGG [SEQ ID NO:25]

Oligo #5

CATGGCTAAC TGCTCTAACA TGAT [SEQ ID NO: 151]

Oligo #6

CGATCAT GTTAGAGCAGTTAGC [SEQ ID NO: 152]

Oligo #7 IL3MUTNCO

TGTCTGCTCA GGCCATGGCT [SEQ ID NO:26]

Oligo #8 IL3T93

GCGCGAATTC ATTCCAGTCA CCGTCCTTGA TATGGTCGAC TTATTACGTG GGTGCGGCCG TGGCTAG [SEQ ID NO:27]

Oligo #9 IL3T97

GCGCGAATTC ATTCCAGTCA CCGTCGACTT ATTAGATTGG ATGTCGCGTG GGTGC [SEQ

ID NO:28]

Oligo #10 IL3T101

GCGCGAATTC GTCGACTTAT TAGTCCTTGA TATGGATTGG ATG [SEQ ID NO:31]

Oligo #11 IL3R94

GCGCGAATTC ATTCCAGTCA CCGTCCTTGA TATGGATTGG ATGSNNCGTG GGTGCGGCCG TGGCTAG [SEQ ID NO:32]

Oligo #12 IL3R95

GCGCGAATTC ATTCCAGTCA CCGTCCTTGA TATGGATTGG SNNTCGCGTG GGTGCGGCCG TGGC [SEQ ID NO:33]

Oligo #13 IL3R96

GCGCGAATTC ATTCCAGTCA CCGTCCTTGA TATGGATSNN ATGTCGCGTG GGTGCGGCCG T [SEQ ID NO:34]

Oligo #14 IL3R97

GCGCGAATTC ATTCCAGTCA CCGTCCTTGA TATGSNNTGG ATGTCGCGTG GGTGCGGC [SEQ ID NO:35]

Oligo #15 IL3P9497

GATATGGATT GGATGTCGCG TGGG [SEQ ID NO:36]

Oligo #16 IL3R98

GCGCGAATTC ATTCCAGTCA CCGTCCTTGA TSNNGATTGG ATGTCGCGTG GGTGC [SEQ ID NO:37]

Oligo #17 IL3R99

GCGCGAATTC ATTCCAGTCA CCGTCCTTSN NATGGATTGG ATGTCGCGTG GG [SEQ ID NO:38]

Oligo #18 IL3R100

GCGCGAATTC ATTCCAGTCA CCGTCSNNGA TATGGATTGG ATGTCGCGT [SEQ ID NO:39]

Oligo #19 IL3R101

GCGCGAATTC ATTCCAGTCA CCSNNCTTGA TATGGATTGG ATGTCG [SEQ ID NO:40]

Oligo #20 IL3P98100

GTCACCGTCC TTGATATGGA TTGG [SEQ ID NO:41]

Oligo #21 IL3R102

GCGCGAATTC ATTCCAGTCS NNGTCCTTGA TATGGATTGG ATG [SEQ ID NO:42]

Oligo #22 IL3R103

GCGCGAATTC ATTCCASNNA CCGTCCTTGA TATGGATTGG [SEQ ID NO:43]

Oligo #23 IL3R104

GCGCGAATTC ATTSNNGTCA CCGTCCTTGA TATGGAT [SEQ ID NO:44]

Oligo #24 IL3R105

GCGCGAATTC SNNCCAGTCA CCGTCCTTGA TATG [SEQ ID NO:45]

Oligo #25 IL3P102105

GAATTCATTC CAGTCACCGT TCCTT [SEQ ID NO:46]

Oligo #26 IL3MUTR1

CGCGCGGAAT TCATTCCAGT CACCGT [SEQ ID NO:47]

Oligo #27 DEL1722

CGCGCGCCAT GGCTAACTGC ATTATAACAC ACACTTAAAG CA [SEQ ID NO:48]

Oligo #28 DEL2328

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAACA GCCACCTTTG CCTTTGCT [SEQ ID NO:49]

Oligo #29 DEL2934

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACACAC TTAAAGCTGG ACTTCAACAA CCTCAA [SEQ ID NO:50]

Oligo #30 DEL3540

GCGCGCGATA TCTTGGTCTT CTTCACCATT CAGCGGCAGC GGTGGCTGCT [SEQ ID NO:51]

Oligo #31 DEL4146

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCCATC AGGATGAGGT TGTTGAAGTC CAGCA [SEQ ID NO:52]

Oligo #32 DEL4752

GCGCGCCTCG AGGTTTGGAC GACGAAGATC TTGGTCTTCA CCATTGA [SEQ ID NO:53]

Oligo #33 DEL5358

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTG AATGCCTCGT TATTTTCCAT CAGGATAT [SEQ ID NO:54]

Oligo #34 DEL5964

GCGCGCTGAT GCATTCTGCA GAGACTTGAC GAGGTTTGGA CGACGAAGGT [SEQ ID NO:55]

Oligo #35 DEL6570

GCGCGCCTCG AGGCATTCAA CCGTGCTGCA TCAGCAATTG AGAGCAT [SEQ ID NO:56]

Oligo #36 DEL7176

GCGCGCCTGC AGAATATTCT TAAAAATCTC CTGCC [SEQ ID NO:57]

Oligo #37 DEL7782

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCCCATGTC TGCCGCTAGC CAC [SEQ ID NO:58]

Oligo #38 DEL8388

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AAAATCTCCT GACGGCCGCA CCCACGCGAC A [SEQ ID NO:59]

Oligo #39 DEL8893

CGCGCGGAAT TCATTCCAGT CACCGTCCTT GATATGGATT GGATGTCGCA GGGCAGACAT GGCAGGA [SEQ ID NO:60]

Oligo #40 DEL106111

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTCTCCAA GGTTTTCAGA TAGAAGGTAT TCCAGTCACC GTCCTTGA [SEQ ID NO:61]

Oligo #41 DEL112117

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTCTCCAA CAGTTTACGA CGGAATTCAT [SEQ ID NO:62]

Oligo #42 DEL118123

CGCGCGAAGC TTATTACTGT TGGGTTTTCA GATAGAAGGT CA [SEQ ID NO:63]

Oligo #43 R17IL3 Length: 000058

CGCGCGCCAT GGCTAACTGC NNSAACATGA TCGATGAAAT TATAACACAC TTAAAGCA [SEQ ID NO:64]

Oligo #44 R18IL3 Length: 000058

CGCGCGCCAT GGCTAACTGC TCTNNSATGA TCGATGAAAT TATAACACAC TTAAAGCA [SEQ ID NO:222]

Oligo #45 R19IL3 Length: 000058

CGCGCGCCAT GGCTAACTGC TCTAACNNSA TCGATGAAAT TATAACACAC TTAAAGCA
[SEQ ID NO:223]

Oligo #46 R20IL3 Length: 000058

CGCGCGCCAT GGCTAACTGC TCTAACATGN NSGATGAAAT TATAACACAC TTAAAGCA
[SEQ ID NO:224]

Oligo #47 R21IL3 Length: 000058

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCNNSGAAAT TATAACACAC TTAAAGCA
[SEQ ID NO:225]

Oligo #48 R22IL3 Length: 000058

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATNNSAT TATAACACAC TTAAAGCA
[SEQ ID NO:226]

Oligo #49 R23IL3 Length: 000076

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAANN SATAACACAC TTAAAGCAGC
CACCTTTGCC TTTGCT [SEQ ID NO:227]

Oligo #50 R24IL3 Length: 000076

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TNNSACACAC TTAAAGCAGC
CACCTTTGCC TTTGCT [SEQ ID NO:228]

Oligo #51 R25IL3 Length: 000076

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATANNSCAC TTAAAGCAGC
CACCTTTGCC TTTGCT [SEQ ID NO:229]

Oligo #52 R26IL3 Length: 000076

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACANNS TTAAAGCAGC
CACCTTTGCC TTTGCT [SEQ ID NO:74]

Oligo #53 R27IL3 Length: 000076

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACACAC NNSAAGCAGC
CACCTTTGCC TTTGCT [SEQ ID NO:75]

Oligo #54 R28IL3 Length: 000076

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACACAC TTANNSCAGC
CACCTTTGCC TTTGCT [SEQ ID NO:76]

Oligo #55 R29IL3 Length: 000094

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACACAC TTAAAGNNSC
CACCTTTGCC TTTGCTGGAC TTCAACAACC TCAA [SEQ ID NO:77]

Oligo #56 R30IL3 Length: 000094

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACACAC TTAAAGCAGN
NSCCTTTGCC TTTGCTGGAC TTCAACAACC TCAA [SEQ ID NO:78]

Oligo #57 R31IL3 Length: 000094

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACACAC TTAAAGCAGC
CANNSTTGCC TTTGCTGGAC TTCAACAACC TCAA [SEQ ID NO:79]

Oligo #58 R32IL3 Length: 000094

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACACAC TTAAAGCAGC
CACCTNNSCC TTTGCTGGAC TTCAACAACC TCAA [SEQ ID NO:80]

Oligo #59 R33IL3 Length: 000094

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACACAC TTAAAGCAGC
CACCTTTGNN STTGCTGGAC TTCAACAACC TCAA [SEQ ID NO:81]

Oligo #60 R34IL3 Length: 000094

CGCGCGCCAT GGCTAACTGC TCTAACATGA TCGATGAAAT TATAACACAC TTAAAGCAGC
CACCTTTGCC TNNSCTGGAC TTCAACAACC TCAA [SEQ ID NO:82]

Oligo #61 R35IL3 Length: 000065

GCGCGCGATA TCTTGGTCTT CACCATTGAG GTTGTTGAAG TCSNNCAGCG GCAGCGGTGG
CTGCT [SEQ ID NO:83]

Oligo #62 R36IL3 Length: 000065

GCGCGCGATA TCTTGGTCTT CACCATTGAG GTTGTTGAAS NNCAGCAGCG GCAGCG
GTGGCTGCT [SEQ ID NO:84]

Oligo #63 R37IL3 Length: 000065

GCGCGCGATA TCTTGGTCTT CACCATTGAG GTTGTTSNNG TCCAGCAGCG GCAGCGGTGG
CTGCT [SEQ ID NO:85]

Oligo #64 R38IL3 Length: 000065

GCGCGCGATA TCTTGGTCTT CACCATTGAG GTTSNNGAAG TCCAGCAGCG GCAGCGGTGG
CTGCT [SEQ ID NO:86]

Oligo #65 R39IL3 Length: 000065

GCGCGCGATA TCTTGGTCTT CACCATTGAG SNNGTTGAAG TCCAGCAGCG GCAGCGGTGG CTGCT [SEQ ID NO:87]

Oligo #66 R40IL3 Length: 000065

GCGCGCGATA TCTTGGTCTT CACCATTSNN GTTGTTGAAG TCCAGCAGCG GCAGCGGTGG CTGCT [SEQ ID NO:88]

Oligo #67 R41IL3 Length: 000083

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCCATC AGGATATCTT GGTCTTCACC SNNGAGGTTG TTGAAGTCCA GCA [SEQ ID NO:89]

Oligo #68 R42IL3 Length: 000083

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCCATC AGGATATCTT GGTCTTCSNN ATTGAGGTTG TTGAAGTCCA GCA [SEQ ID NO:90]

Oligo #69 R43IL3 Length: 000083

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCCATC AGGATATCTT GGTCSNNACC ATTGAGGTTG TTGAAGTCCA GCA [SEQ ID NO:91]

Oligo #70 R44IL3 Length: 000083

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCCATC AGGATATCTT GSNNTTCACC ATTGAGGTTG TTGAAGTCCA GCA [SEQ ID NO:92]

Oligo #71 R45IL3 Length: 000083

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCCATC AGGATATCSN NGTCTTCACC ATTGAGGTTG TTGAAGTCCA GCA [SEQ ID NO:93]

Oligo #72 R46IL3 Length: 000083

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCCATC AGGATSNNTT GGTCTTCACC ATTGAGGTTG TTGAAGTCCA GCA [SEQ ID NO:94]

Oligo #73 R47IL3 Length: 000065

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCCATC AGSNNATCTT GGTCTTCACC ATTGA [SEQ ID NO:95]

Oligo #74 R48IL3 Length: 000065

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCCATS NNGATATCTT GGTCTTCACC ATTGA [SEQ ID NO:96]

Oligo #75 R49IL3 Length: 000065

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTTTCSNNC AGGATATCTT GGTCTTCACC ATTGA [SEQ ID NO:97]

Oligo #76 R50IL3 Length: 000065

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT ATTSNNCATC AGGATATCTT GGTCTTCACC ATTGA [SEQ ID NO:98]

Oligo #77 R51IL3 Length: 000065

GCGCGCCTCG AGGTTTGGAC GACGAAGGTT SNNTTCCATC AGGATATCTT GGTCTTCACC ATTGA [SEQ ID NO:99]

Oligo #78 R52IL3 Length: 000065

GCGCGCCTCG AGGTTTGGAC GACGAAGSNN ATTTTCCATC AGGATATCTT GGTCTTCACC ATTGA [SEQ ID NO:100]

Oligo #79 R53IL3 Length: 000086

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTG AATGCCTCGA GGTTTGGACG ACGSNNGTTA TTTTCCATCA GGATAT [SEQ ID NO:101]

Oligo #80 R54IL3 Length: 000086

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTG AATGCCTCGA GGTTTGGACG SNNAAGGTTA TTTTCCATCA GGATAT [SEQ ID NO:102]

Oligo #81 R55IL3 Length: 000086

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTG AATGCCTCGA GGTTTGGSNN ACGAAGGTTA TTTTCCATCA GGATAT [SEQ ID NO:103]

Oligo #82 R56IL3 Length: 000086

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTG AATGCCTCGA GGTTSNNACG ACGAAGGTTA TTTTCCATCA GGATAT [SEQ ID NO:104]

Oligo #83 R57IL3 Length: 000086

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTG AATGCCTCGA GSNNTGGACG ACGAAGGTTA TTTTCCATCA GGATAT [SEQ ID NO:105]

Oligo #84 R58IL3 Length: 000086

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTG AATGCCTCSN NGTTTGGACG ACGAAGGTTA TTTTCCATCA GGATAT [SEQ ID NO:106]

Oligo #85 R59IL3 Length: 000068

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTG AATGCSNNGA GGTTTGGACG ACGAAGGT [SEQ ID NO:107]

Oligo #86 R60IL3 Length: 000068

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTG AASNNCTCGA GGTTTGGACG ACGAAGGT [SEQ ID NO:108]

Oligo #87 R61IL3 Length: 000068

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGGTTS NNTGCCTCGA GGTTTGGACG ACGAAGGT [SEQ ID NO:109]

Oligo #88 R62IL3 Length: 000068

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCACGSNNG AATGCCTCGA GGTTTGGACG ACGAAGGT [SEQ ID NO:110]

Oligo #89 R63IL3 Length: 000068

GCGCGCTGAT GCATTCTGCA GAGACTTGAC AGCSNNGTTG AATGCCTCGA GGTTTGGACG ACGAAGGT [SEQ ID NO:111]

Oligo #90 R64IL3 Length: 000068

GCGCGCTGAT GCATTCTGCA GAGACTTGAC SNNACGGTTG AATGCCTCGA GGTTTGGACG ACGAAGGT [SEQ ID NO:112]

Oligo #91 R65IL3 Length: 000065

GCGCGCCTCG AGGCATTCAA CCGTGCTNNS AAGTCTCTGC AGAATGCATC AGCAATTGAG AGCAT [SEQ ID NO:113]

Oligo #92 R66IL3 Length: 000065

GCGCGCCTCG AGGCATTCAA CCGTGCTGTC NNSTCTCTGC AGAATGCATC AGCAATTGAG AGCAT [SEQ ID NO:114]

Oligo #93 R67IL3 Length: 000065

GCGCGCCTCG AGGCATTCAA CCGTGCTGTC AAGNNSCTGC AGAATGCATC AGCAATTGAG AGCAT [SEQ ID NO:115]

Oligo #94 R68IL3 Length: 000065

GCGCGCCTCG AGGCATTCAA CCGTGCTGTC AAGTCTNNSC AGAATGCATC AGCAATTGAG AGCAT [SEQ ID NO:116]

Oligo #95 R69IL3 Length: 000065

GCGCGCCTCG AGGCATTCAA CCGTGCTGTC AAGTCTCTGN NSAATGCATC AGCAATTGAG AGCAT [SEQ ID NO:117]

Oligo #96 R70IL3 Length: 000065

GCGCGCCTCG AGGCATTCAA CCGTGCTGTC AAGTCTCTGC AGNNSGCATC AGCAATTGAG AGCAT [SEQ ID NO:118]

Oligo #97 R71IL3 Length: 000053

GCGCGCCTGC AGAATNNSTC AGCAATTGAG AGCATTCTTA AAAATCTCCT GCC [SEQ ID NO:119]

Oligo #98 R72IL3 Length: 000053

GCGCGCCTGC AGAATGCANN SGCAATTGAG AGCATTCTTA AAAATCTCCT GCC [SEQ ID NO:120]

Oligo #99 R73IL3 Length: 000053

GCGCGCCTGC AGAATGCATC ANNSATTGAG AGCATTCTTA AAAATCTCCT GCC [SEQ ID NO:121]

Oligo #100 R74IL3 Length: 000053

GCGCGCCTGC AGAATGCATC AGCANNSGAG AGCATTCTTA AAAATCTCCT GCC [SEQ ID NO:122]

Oligo #101 R75IL3 Length: 000053

GCGCGCCTGC AGAATGCATC AGCAATTNNS AGCATTCTTA AAAATCTCCT GCC [SEQ ID NO:123]

Oligo #102 R76IL3 Length: 000053

GCGCGCCTGC AGAATGCATC AGCAATTGAG NNSATTCTTA AAAATCTCCT GCC [SEQ ID NO:124]

Oligo #103 R77IL3 Length: 000071

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCNNSCTTA AAAATCTCCT GCCATGTCTG CCGCTAGCCA C [SEQ ID NO:125]

Oligo #104 R78IL3 Length: 000071

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTNNSA AAAATCTCCT GCCATGTCTG CCGCTAGCCA C [SEQ ID NO:126]

Oligo #105 R79IL3 Length: 000071

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTN NSAATCTCCT GCCATGTCTG CCGCTAGCCA C [SEQ ID NO:127]

Oligo #106 R80IL3 Length: 000071

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AANNSCTCCT GCCATGTCTG CCGCTAGCCA C [SEQ ID NO:138]

Oligo #107 R81IL3 Length: 000071

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AAAATNNSCT GCCATGTCTG CCGCTAGCCA C [SEQ ID NO:139]

Oligo #108 R82IL3 Length: 000071

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AAAATCTCNN SCCATGTCTG
CCGCTAGCCA C [SEQ ID NO:140]

Oligo #109 R83IL3 Length: 000089

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AAAATCTCCT GNNSTGTCTG
CCGCTAGCCA CGGCCGCACC CACGCGACA [SEQ ID NO:141]

Oligo #110 R84IL3 Length: 000089

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AAAATCTCCT GCCANNSCTG
CCGCTAGCCA CGGCCGCACC CACGCGACA [SEQ ID NO:142]

Oligo #111 R85IL3 Length: 000089

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AAAATCTCCT GCCATGTNNS
CCGCTAGCCA CGGCCGCACC CACGCGACA [SEQ ID NO:143]

Oligo #112 R86IL3 Length: 000089

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AAAATCTCCT GCCATGTCTG
NNSCTAGCCA CGGCCGCACC CACGCGACA [SEQ ID NO:157]

Oligo #113 R87IL3 Length: 000089

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AAAATCTCCT GCCATGTCTG
CCGNNSGCCA CGGCCGCACC CACGCGACA [SEQ ID NO:158]

Oligo #114 R88IL3 Length: 000089

GCGCGCCTGC AGAATGCATC AGCAATTGAG AGCATTCTTA AAAATCTCCT GCCATGTCTG
CCGCTANNSA CGGCCGCACC CACGCGACA [SEQ ID NO:159]

Oligo #115 R89IL3 Length: 000086

CGCGCGGAAT TCATTCCAGT CACCGTCCTT GATATGGATT GGATGTCGCG TGGGTGCGGC
SNNGGCCAGG GGCAGACATG GCAGGA [SEQ ID NO:160]

Oligo #116 R90IL3 Length: 000086

CGCGCGGAAT TCATTCCAGT CACCGTCCTT GATATGGATT GGATGTCGCG TGGGTGCSNN
CGTGGCCAGG GGCAGACATG GCAGGA [SEQ ID NO:161]

Oligo #117 R91IL3 Length: 000086

CGCGCGGAAT TCATTCCAGT CACCGTCCTT GATATGGATT GGATGTCGCG TGGGSNNGGC
CGTGGCCAGG GGCAGACATG GCAGGA [SEQ ID NO:162]

Oligo #118 R92IL3 Length: 000086

CGCGCGGAAT TCATTCCAGT CACCGTCCTT GATATGGATT GGATGTCGCG TSNNTGCGGC
CGTGGCCAGG GGCAGACATG GCAGGA [SEQ ID NO:163]

Oligo #119 R93IL3 Length: 000086

CGCGCGGAAT TCATTCCAGT CACCGTCCTT GATATGGATT GGATGTCGSN NGGGTGCGGC
CGTGGCCAGG GGCAGACATG GCAGGA [SEQ ID NO:164]

Oligo #120 3PR106 Length: 000048

TTTCAGATAG AAGGTCAGTT TACGACGGAA SNNATTCCAG TCACCGTC [SEQ ID NO:165]

Oligo #121 3PR107 Length: 000048

TTTCAGATAG AAGGTCAGTT TACGACGSNN TTCATTCCAG TCACCGTC [SEQ ID NO:166]

Oligo #122 3PR108 Length: 000048

TTTCAGATAG AAGGTCAGTT TACGSNNGAA TTCATTCCAG TCACCGTC [SEQ ID NO:167]

Oligo #123 3PR109 Length: 000048

TTTCAGATAG AAGGTCAGTT TSNNACGGAA TTCATTCCAG TCACCGTC [SEQ ID NO:168]

Oligo #124 3PR110 Length: 000048

TTTCAGATAG AAGGTCAGSN NACGACGGAA TTCATTCCAG TCACCGTC [SEQ ID NO:169]

Oligo #125 3PR111 Length: 000048

TTTCAGATAG AAGGTSNNTT TACGACGGAA TTCATTCCAG TCACCGTC [SEQ ID NO:170]

Oligo #126 IL3MUTD3 Length: 000023

CGCGCGAAGC TTATTACTGT TGA [SEQ ID NO:171]

Oligo #127 R112IL3 Length: 000078

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTCTCCAA GGTTTTCAGA TAGAASNNCA
GTTTACGACG GAATTCAT [SEQ ID NO:172]

Oligo #128 R113IL3 Length: 000078

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTCTCCAA GGTTTTCAGA TASNNGGTCA
GTTTACGACG GAATTCAT [SEQ ID NO:173]

Oligo #129 R114IL3 Length: 000078

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTCTCCAA GGTTTTCAGS NNGAAGGTCA
GTTTACGACG GAATTCAT [SEQ ID NO:174]

Oligo #130 R115IL3 Length: 000078

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTCTCCAA GGTTTTSNNA TAGAAGGTCA
GTTTACGACG GAATTCAT [SEQ ID NO:175]

Oligo #131 R116IL3 Length: 000078

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTCTCCAA GGTSNNCAGA TAGAAGGTCA
GTTTACGACG GAATTCAT [SEQ ID NO:176]

Oligo #132 R117IL3 Length: 000078

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTCTCCAA SNNTTTCAGA TAGAAGGTCA
GTTTACGACG GAATTCAT [SEQ ID NO:177]

Oligo #133 R118IL3 Length: 000060

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTCTCSNN GGTTTTCAGA TAGAAGGTCA
[SEQ ID NO:178]

Oligo #134 R119IL3 Length: 000060

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CGTTSNNCAA GGTTTTCAGA TAGAAGGTCA
[SEQ ID NO:179]

Oligo #135 R120IL3 Length: 000060

CGCGCGAAGC TTATTACTGT TGAGCCTGCG CSNNCTCCAA GGTTTTCAGA TAGAAGGTCA
[SEQ ID NO:180]

Oligo #136 R121IL3 Length: 000060

CGCGCGAAGC TTATTACTGT TGAGCCTGSN NGTTCTCCAA GGTTTTCAGA TAGAAGGTCA
[SEQ ID NO:181]

Oligo #137 R122IL3 Length: 000060

CGCGCGAAGC TTATTACTGT TGAGCSNNCG CGTTCTCCAA GGTTTTCAGA TAGAAGGTCA
[SEQ ID NO:182]

Oligo #138 R123IL3 Length: 000060

CGCGCGAAGC TTATTACTGT TGSNNCTGCG CGTTCTCCAA GGTTTTCAGA TAGAAGGTCA
[SEQ ID NO:183]

Oligo #139 P1722IL3 Length: 000024

TGCTCTAACA TGATCGATGA AATT [SEQ ID NO:184]

Oligo #140 P2328IL3 Length: 000024

GAAATTATAA CACACTTAAA GCAG [SEQ ID NO:185]

Oligo #141 P2934IL3 Length: 000024

AAGCAGCCAC CTTTGCCTTT GCTG [SEQ ID NO:186]

Oligo #142 P3540IL3 Length: 000024

AAGCAGCCAC CGCTGCCGCT GCTG [SEQ ID NO:187]

Oligo #143 PRB41-46 Length: 000024

CTCAATGGTG AAGACCAAGA TATC [SEQ ID NO:188]

Oligo #144 PRB47-52 Length: 000024

GATATCCTGA TGGAAAATAA CCTT [SEQ ID NO:189]

Oligo #145 PRB53-58 Length: 000024

AACCTTCGTC GTCCAAACCT CGAG [SEQ ID NO:190]

Oligo #146 PRB59-64 Length: 000024

CTCGAGGCAT TCAACCGTGC TGTC [SEQ ID NO:191]

Oligo #147 PRB65-70 Length: 000024

GCTGTCAAGT CTCTGCAGAA TGCA [SEQ ID NO:192]

Oligo #148 P7176IL3 Length: 000024

AATGCATCAG CAATTGAGAG CATT [SEQ ID NO:193]

Oligo #149 P7782IL3 Length: 000024

AGCATTCTTA AAAATCTCCT GCCA [SEQ ID NO:194]

Oligo #150 P8388IL3 Length: 000024

CTGCCATGTC TGCCCCTGGC CACG [SEQ ID NO:195]

Oligo #151 P8893IL3 Length: 000024

CTGGCCACGG CCGCACCCAC GCGA [SEQ ID NO:196]

Oligo #152 P106111 Length: 000024

AATGAATTCC GTCGTAAACT GACC [SEQ ID NO:197]

Oligo #153 P112117 Length: 000024

CTGACCTTCT ATCTGAAAAC CTTG [SEQ ID NO:198]

Oligo #154 P118123 Length: 000024

ACCTTGGAGA ACGCGCAGGC TCAA [SEQ ID NO:199]

Oligo #155 PSTECRI1.REQ Length: 000022

GAATGCATCA GCAATTGAGA GC [SEQ ID NO:200]

Oligo #156 PSTECRI5.REQ Length: 000020

AATTGCTGAT GCATTCTGCA [SEQ ID NO:201]

Oligo #157 PSTECRI2.REQ Length: 000024

ATTCTTAAAA ATCTCCTGCC ATGT [SEQ ID NO:202]

Oligo #158 PSTECRI6.REQ Length: 000024

CAGGAGATTT TTAAGAATGC TCTC [SEQ ID NO:203]

Oligo #159 PSTECRI3.REQ Length: 000030

CTGCCCCTGG CCACGGCCGC ACCCACGCGA [SEQ ID NO:204]

Oligo #160 PSTECRI7.REQ Length: 000030

GGGTGCGGCC GTGGCCAGGG GCAGACATGG [SEQ ID NO:205]

Oligo #161 98I100R4.REQ Length: 000034

CATCCAATCA TCATCCGTGA CGGTGACTGG AATG [SEQ ID NO:206]

Oligo #162 98I100R8.REQ Length: 000044

AATTCATTCC AGTCACCGTC ACGGATGATG ATTGGATGTC GCGT [SEQ ID NO:207]

Oligo #163 95R8I0R4.REQ Length: 000034

CGCCCAATCA TCATCCGTGA CGGTGACTGG AATG [SEQ ID NO:208]

Oligo #164 95R8I0R8.REQ Length: 000044

AATTCATTCC AGTCACCGTC ACGGATGATG ATTGGGCGTC GCGT [SEQ ID NO:209]

Oligo #165 NCOECRV1.REQ Length: 000040

CATGGCTAAC TGCTCTAACA TGATCGATGA AATTATAACA [SEQ ID NO:210]

Oligo #166 NCOECRV4.REQ Length: 000045

CTTTAAGTGT GTTATAATTT CATCGATCAT GTTAGAGCAG TTAGC [SEQ ID NO:211]

Oligo #167 NCOECRV2.REQ Length: 000036

CACTTAAAGC AGCCACCTTT GCCTTTGCTG GACTTC [SEQ ID NO:212]

Oligo #168 NCOECRV5.REQ Length: 000036

GAGGTTGTTG AAGTCCAGCA AAGGCAAAGG TGGCTG [SEQ ID NO:213]

Oligo #169 2D5M6SUP.REQ Length: 000027

AACAACCTCA ATGACGAAGA CATGTCT [SEQ ID NO:214]

Oligo #170 2D5M6SLO.REQ Length: 000018

AGACATGTCT TCGTCATT [SEQ ID NO:215]

Oligo #15(A) Length: 000016

TGAACCATAT GTCAGG [SEQ ID NO:29]

Oligo #16(A) Length: 000024

AATTCCTGAC ATATGGTTCA TGCA [SEQ ID NO:30]

Oligo #51(A) Length: 000034

GCCGATACCGCGGCATACTCCCACCATTCAGAGA [SEQ ID NO:155]

Oligo #52(A) Length: 000033

GCCGATAAGATCTAAAACGGGTATGGAGAAACA [SEQ ID NO:156]

Oligo #171 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCAACGA AATTATAACA [SEQ. ID NO: 69]

Oligo #172 Length: 000045

CTTTAAGTGT GTTATAATTT CGTTGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:70]

Oligo #173 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCCAAGA AATTATAACA [SEQ. ID NO:71

Oligo #174 Length: 000045

CTTTAAGTGT GTTATAATTT CTTGGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:72]

Oligo #175 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCGAAGA AATTATAACA [SEQ. ID NO:73]

Oligo #176 Length: 000045

CTTTAAGTGT GTTATAATTT CTTCGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:219]

Oligo #177 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCAGCGA AATTATAACA [SEQ. ID NO:230]

Oligo #178 Length: 000045

CTTTAAGTGT GTTATAATTT CGCTGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:231]

Oligo #179 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCACCGA AATTATAACA [SEQ. ID NO:232]

Oligo #180 Length: 000045

CTTTAAGTGT GTTATAATTT CCGTGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:233]

Oligo #181 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCGATAA CATTATAACA [SEQ. ID NO:234]

Oligo #182 Length: 000045

CTTTAAGTGT GTTATAATGT TATCGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:235]

Oligo #183 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCGATGA CATTATAACA [SEQ. ID NO:236]

Oligo #184 Length: 000045

CTTTAAGTGT GTTATAATGT CATCGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:237]

Oligo #185 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCGATCA GATTATAACA [SEQ. ID NO:238]

Oligo #186 Length: 000045

CTTTAAGTGT GTTATAATCT GATCGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:239]

Oligo #187 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCGATCT GATTATAACA [SEQ. ID NO:240]

Oligo #188 Length: 000045

CTTTAAGTGT GTTATAATCA GATCGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:241]

Oligo #189 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCGATGT TATTATAACA [SEQ. ID NO:242]

Oligo #190 Length: 000045

CTTTAAGTGT GTTATAATAA CATCGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:243]

Oligo #191 Length: 000036

CACTTAAAGC AGCCACCTTT GCCTGCTCTG GACTTC [SEQ. ID NO:244]

Oligo #192 Length: 000036

GAGGTTGTTG AAGTCCAGAG CAGGCAAAGG TGGCTG [SEQ. ID NO:245]

Oligo #193 Length: 000036

CACTTAAAGC AGCCACCTTT GCCTCGTCTG GACTTC [SEQ. ID NO:246]

Oligo #194 Length: 000036

GAGGTTGTTG AAGTCCAGAC GAGGCAAAGG TGGCTG [SEQ. ID NO:247]

Oligo #195 Length: 000036

CACTTAAAGC AGCCACCTTT GCCTCAGCTG GACTTC [SEQ. ID NO:248]

Oligo #196 Length: 000036

GAGGTTGTTG AAGTCCAGCT GAGGCAAAGG TGGCTG [SEQ. ID NO:249]

Oligo #197 Length: 000036

CACTTAAAGC AGCCACCTTT GCCTGAACTG GACTTC [SEQ. ID NO:250]

Oligo #198 Length: 000036

GAGGTTGTTG AAGTCCAGCT CAGGCAAAGG TGGCTG [SEQ. ID NO:251]

Oligo #199 Length: 000036

CACTTAAAGC AGCCACCTTT GCCTATCCTG GACTTC [SEQ. ID NO:252]

Oligo #200 Length: 000036

GAGGTTGTTG AAGTCCAGGA TAGGCAAAGG TGGCTG [SEQ. ID NO:253]

Oligo #201 Length: 000036

CACTTAAAGC AGCCACCTTT CCCTTTCCTG GACTTC [SEQ. ID NO:254]

Oligo #202 Length: 000036

GAGGTTGTTG AAGTCCAGGA AAGGCAAAGG TGGCTG [SEQ. ID NO:255]

Oligo #203 Length: 000036

CACTTAAAGC AGCCACCTTT GCCTACCCTG GACTTC [SEQ. ID NO:256]

Oligo #204 Length: 000036

GAGGTTGTTG AAGTCCAGGG TAGGCAAAGG TGGCTG [SEQ. ID NO:257]

Oligo #205 Length: 000027

AACAACCTCA ATCGTGAAGA CCAAGAT [SEQ. ID NO:258]

Oligo #206 Length: 000018

ATCTTGGTCT TCACGATT [SEQ. ID NO:259]

Oligo #207 Length: 000027

AACAACCTCA ATAACGAAGA CCAAGAT [SEQ. ID NO:260]

Oligo #208 Length: 000018

ATCTTGGTCT TCGTTATT [SEQ. ID NO:261]

Oligo #209 Length: 000027

AACAACCTCA ATGAAGAAGA CCAAGAT [SEQ. ID NO:262]

Oligo #210 Length: 000018

ATCTTGGTCT TCTTCATT [SEQ. ID NO:263]

Oligo #211 Length: 000027

AACAACCTCA ATATCGAAGA CCAAGAT [SEQ. ID NO:264]

Oligo #212 Length: 000018

ATCTTGGTCT TCGATATT [SEQ. ID NO:265]

Oligo #213 Length: 000027

AACAACCTCA ATCTGGAAGA CCAAGAT [SEQ. ID NO:266]

Oligo #214 Length: 000018

ATCTTGGTCT TCCAGATT [SEQ. ID NO:267]

Oligo #215 Length: 000027

AACAACCTCA ATAAAGAAGA CCAAGAT [SEQ. ID NO:268]

Oligo #216 Length: 000018

ATCTTGGTCT TCTTTATT [SEQ. ID NO:269]

Oligo #217 Length: 000027

AACAACCTCA ATATGGAAGA CCAAGAT [SEQ. ID NO:270]

Oligo #218 Length: 000018

ATCTTGGTCT TCCATATT [SEQ. ID NO:271]

Oligo #219 Length: 000027

AACAACCTCA ATTTCGAAGA CCAAGAT [SEQ. ID NO:272]

Oligo #220 Length: 000018

ATCTTGGTCT TCGAAATT [SEQ. ID NO:273]

Oligo #221 Length: 000027

AACAACCTCA ATACCGAAGA CCAAGAT [SEQ. ID NO:274]

Oligo #222 Length: 000018

ATCTTGGTCT TCGGTATT [SEQ. ID NO:275]

Oligo #223 Length: 000027

AACAACCTCA ATTACGAAGA CCAAGAT [SEQ. ID NO:276]

Oligo #224 Length: 000018

ATCTTGGTCT TCGTAATT [SEQ. ID NO:277]

Oligo #225 Length: 000027

AACAACCTCA ATGTTGAAGA CCAAGAT [SEQ. ID NO:278]

Oligo #226 Length: 000018

ATCTTGGTCT TCAACATT [SEQ. ID NO:279]

Oligo #227 Length: 000027

AACAACCTCA ATGGGCGTGA CCAAGAT [SEQ. ID NO:280]

Oligo #228 Length: 000018

ATCTTGGTCT CGCCCATT [SEQ. ID NO:281]

Oligo #229 Length: 000027

AACAACCTCA ATGGGCAGGA CCAAGAT [SEQ. ID NO:282]

Oligo #230 Length: 000018

ATCTTGGTCC TGCCCATT [SEQ. ID NO:283]

Oligo #231 Length: 000027

AACAACCTCA ATGGGGGTGA CCAAGAT [SEQ. ID NO:284]

Oligo #232 Length: 000018

ATCTTGGTCA CCCCCATT [SEQ. ID NO:285]

Oligo #233 Length: 000027

AACAACCTCA ATGGGACCGA CCAAGAT [SEQ. ID NO:286]

Oligo #234 Length: 000018

ATCTTGGTCG GTCCCATT [SEQ. ID NO:287]

Oligo #235 Length: 000027

AACAACCTCA ATGGGGAAGC TCAAGAT [SEQ. ID NO:288]

Oligo #236 Length: 000018

ATCTTGAGCT TCCCCATT [SEQ. ID NO:289]

Oligo #237 Length: 000027

AACAACCTCA ATGGGGAAAA CCAAGAT [SEQ. ID NO:290]

Oligo #238 Length: 000018

ATCTTGGTTT TCCCCATT [SEQ. ID NO:291]

Oligo #239 Length: 000027

AACAACCTCA ATGGGGAACA GCAAGAT [SEQ. ID NO:292]

Oligo #240 Length: 000018

ATCTTGCTGT TCCCCATT [SEQ. ID NO:293]

Oligo #241 Length: 000027

AACAACCTCA ATGGGGAAGA ACAAGAT [SEQ. ID NO:294]

Oligo #242 Length: 000018

ATCTTGTTCT TCCCCATT [SEQ. ID NO:295]

Oligo #243 Length: 000027

AACAACCTCA ATGGGGAAGA CGCTGAT [SEQ. ID NO:296]

Oligo #244 Length: 000018

ATCAGCGTCT TCCCCATT [SEQ. ID NO:297]

Oligo #245 Length: 000027

AACAACCTCA ATGGGGAAGA CCGTGAT [SEQ. ID NO:298]

Oligo #246 Length: 000018

ATCACGGTCT TCCCCATT [SEQ. ID NO:299]

Oligo #247 Length: 000027

AACAACCTCA ATGGGGAAGA CAACGAT [SEQ. ID NO:300]

Oligo #248 Length: 000018

ATCGTTGTCT TCCCCATT [SEQ. ID NO:301]

Oligo #249 Length: 000027

AACAACCTCA ATGGGGAAGA CGACGAT [SEQ. ID NO:302]

Oligo #250 Length: 000018

ATCGTCGTCT TCCCCATT [SEQ. ID NO:303]

Oligo #251 Length: 000027

AACAACCTCA ATGGTGAAGA CGAAGAT [SEQ. ID NO:304]

Oligo #252 Length: 000018

ATCTTCGTCT TCCCCATT [SEQ. ID NO:305]

Oligo #253 Length: 000027

AACAACCTCA ATGGTGAAGA CCACGAT [SEQ. ID NO:306]

Oligo #254 Length: 000018

ATCGTGGTCT TCCCCATT [SEQ. ID NO:307]

Oligo #255 Length: 000027

AACAACCTCA ATGGGGAAGA CATCGAT [SEQ. ID NO:308]

Oligo #256 Length: 000018

ATCGATGTCT TCCCCATT [SEQ. ID NO:309]

Oligo #257 Length: 000027

AACAACCTCA ATGGGGAAGA CTCCGAT [SEQ. ID NO:310]

Oligo #258 Length: 000018

ATCGGAGTCT TCCCCATT [SEQ. ID NO:311]

Oligo #259 Length: 000027

AACAACCTCA ATGGGGAAGA CCAAGCT [SEQ. ID NO:312]

Oligo #260 Length: 000018

AGCTTGGTCT TCCCCATT [SEQ. ID NO:313]

Oligo #261 Length: 000027

AACAACCTCA ATGGGGAAGA CCAAAAC [SEQ. ID NO:314]

Oligo #262 Length: 000018

GTTTTGGTCT TCCCCATT [SEQ. ID NO:315]

Oligo #263 Length: 000027

AACAACCTCA ATGGGGAAGA CCAACAG [SEQ. ID NO:316]

Oligo #264 Length: 000018

CTGTTGGTCT TCCCCATT [SEQ. ID NO:317]

Oligo #265 Length: 000027

AACAACCTCA ATGGGGAAGA CCAAGAA [SEQ. ID NO:318]

Oligo #266 Length: 000018

TTCTTGGTCT TCCCCATT [SEQ. ID NO:319]

Oligo #267 Length: 000027

AACAACCTCA ATGGGGAAGA CCAACAC [SEQ. ID NO:320]

Oligo #268 Length: 000018

GTGTTGGTCT TCCCCATT [SEQ. ID NO:321]

Oligo #269 Length: 000027

AACAACCTCA ATGGGGAAGA CCAAATC [SEQ. ID NO:322]

Oligo #270 Length: 000018

GATTTGGTCT TCCCCATT [SEQ. ID NO:323]

Oligo #271 Length: 000027

AACAACCTCA ATGGGGAAGA CCAACTG [SEQ. ID NO:324]

Oligo #272 Length: 000018

CAGTTGGTCT TCCCCATT [SEQ. ID NO:325]

Oligo #273 Length: 000027

AACAACCTCA ATGGGGAAGA CCAAAAA [SEQ. ID NO:326]

Oligo #274 Length: 000018

TTTTTGGTCT TCCCCATT [SEQ. ID NO:327]

Oligo #275 Length: 000027

AACAACCTCA ATGGGGAAGA CCAATAC [SEQ. ID NO:328]

Oligo #276 Length: 000018

GTATTGGTCT TCCCCATT [SEQ. ID NO:329]

Oligo #277 Length: 000027

AACAACCTCA ATGGGGAAGA CCAAGTT [SEQ. ID NO:330]

Oligo #278 Length: 000018

AACTTGGTCT TCCCCATT [SEQ. ID NO:331]

Oligo #279 Length: 000036

ATCGCTATGG AAAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:332]

Oligo #280 Length: 000027

CCTTCGAAGG TTATTTTCCA TAGCGAT [SEQ. ID NO:333]

Oligo #281 Length: 000036

ATCGAAATGG AAAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:334]

Oligo #282 Length: 000027

CCTTCGAAGG TTATTTTCCA TTTCGAT [SEQ. ID NO:335]

Oligo #283 Length: 000036

ATCAAAATGG AAAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:336]

Oligo #284 Length: 000027

CCTTCGAAGG TTATTTTCCA TTTTGAT [SEQ. ID NO:337]

Oligo #285 Length: 000036

ATCATGATGG AAAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:338]

Oligo #286 Length: 000027

CCTTCGAAGG TTATTTTCCA TCATGAT [SEQ. ID NO:339]

Oligo #287 Length: 000036

ATCACCATGG AAAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:340]

Oligo #288 Length: 000027

CCTTCGAAGG TTATTTTCCA TGGTGAT [SEQ. ID NO:341]

Oligo #289 Length: 000036

ATCGTTATGG AAAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:342]

Oligo #290 Length: 000027

CCTTCGAAGG TTATTTTCCA TAACGAT [SEQ. ID NO:343]

Oligo #291 Length: 000036

ATCCTGATGC ACAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:344]

Oligo #292 Length: 000027

CCTTCGAAGG TTATTGTGCA TCAGGAT [SEQ. ID NO:345]

Oligo #293 Length: 000036

ATCCTGATGA TGAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:346]

Oligo #294 Length: 000027

CCTTCGAAGG TTATTCATCA TCAGGAT [SEQ. ID NO:347]

Oligo #295 Length: 000036

ATCCTGATGT TCAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:348]

Oligo #296 Length: 000027

CCTTCGAAGG TTATTGAACA TCAGGAT [SEQ. ID NO:349]

Oligo #297 Length: 000036

ATCCTGATGG CTAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:350]

Oligo #298 Length: 000027

CCTTCGAAGG TTATTAGCCA TCAGGAT [SEQ. ID NO:351]

Oligo #299 Length: 000036

ATCCTGATGA ACAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:352]

Oligo #300 Length: 000027

CCTTCGAAGG TTATTGTTCA TCAGGAT [SEQ. ID NO:353]

Oligo #301 Length: 000036

ATCCTGATGA TCAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:354]

Oligo #302 Length: 000027

CCTTCGAAGG TTATTGATCA TCAGGAT [SEQ. ID NO:355]

Oligo #303 Length: 000036

ATCCTGATGA AAAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:356]

Oligo #304 Length: 000027

CCTTCGAAGG TTATTTTTCA TCAGGAT [SEQ. ID NO:357]

Oligo #305 Length: 000036

ATCCTGATGT CCAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:358]

Oligo #306 Length: 000027

CCTTCGAAGG TTATTGGACA TCAGGAT [SEQ. ID NO:359]

Oligo #307 Length: 000036

ATCCTGATGG TTAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:360]

Oligo #308 Length: 000027

CCTTCGAAGG TTATTAACCA TCAGGAT [SEQ. ID NO:361]

Oligo #309 Length: 000036

ATCCTGATGG AAAATAACCT TGCTAGGCCA AACCTG [SEQ. ID NO:362]

Oligo #310 Length: 000027

CCTAGCAAGG TTATTTTCCA TCAGGAT [SEQ. ID NO:363]

Oligo #311 Length: 000036

ATCCTGATGG AAAATAACCT TAACAGGCCA AACCTG [SEQ. ID NO:364]

Oligo #312 Length: 000027

CCTGTTAAGG TTATTTTCCA TCAGGAT [SEQ. ID NO:365]

Oligo #313 Length: 000036

ATCCTGATGG AAAATAACCT TCACAGGCCA AACCTG [SEQ. ID NO:366]

Oligo #314 Length: 000027

CCTGTGAAGG TTATTTTCCA TCAGGAT [SEQ. ID NO:367]

Oligo #315 Length: 000036

ATCCTGATGG AAAATAACCT TAAAAGGCCA AACCTG [SEQ. ID NO:368]

Oligo #316 Length: 000027

CCTTTTAAGG TTATTTTCCA TCAGGAT [SEQ. ID NO:369]

Oligo #317 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGGCT AACCTG [SEQ. ID NO:370]

Oligo #318 Length: 000024

CCTGTTGAAT GCCTCCAGGT TAGC [SEQ. ID NO:371]

Oligo #319 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGCGT AACCTG [SEQ. ID NO:372]

Oligo #320 Length: 000024

CCTGTTGAAT GCCTCCAGGT TACG [SEQ. ID NO:373]

Oligo #321 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGAAC AACCTG [SEQ. ID NO:374]

Oligo #322 Length: 000024

CCTGTTGAAT GCCTCCAGGT TGTT [SEQ. ID NO:375]

Oligo #323 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGGAA AACCTG [SEQ. ID NO:376]

Oligo #324 Length: 000024

CCTGTTGAAT GCCTCCAGGT TTTC [SEQ. ID NO:377]

Oligo #325 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGCAC AACCTG [SEQ. ID NO:378]

Oligo #326 Length: 000024

CCTGTTGAAT GCCTCCAGGT TGTG [SEQ. ID NO:379]

Oligo #327 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGCTG AACCTG [SEQ. ID NO:380]

Oligo #328 Length: 000024

CCTGTTGAAT GCCTCCAGGT TCAG [SEQ. ID NO:381]

Oligo #329 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGTTC AACCTG [SEQ. ID NO:382]

Oligo #330 Length: 000024

CCTGTTGAAT GCCTCCAGGT TGAA [SEQ. ID NO:383]

Oligo #331 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGACC AACCTG [SEQ. ID NO:384]

Oligo #332 Length: 000024

CCTGTTGAAT GCCTCCAGGT TGGT [SEQ. ID NO:385]

Oligo #333 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGTAC AACCTG [SEQ. ID NO:386]

Oligo #334 Length: 000024

CCTGTTGAAT GCCTCCAGGT TGTA [SEQ. ID NO:387]

Oligo #335 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGGTT AACCTG [SEQ. ID NO:388]

Oligo #336 Length: 000024

CCTGTTGAAT GCCTCCAGGT TAAC [SEQ. ID NO:389]

Oligo #337 Length: 000018

AAAAATCTCG CTCCATGT [SEQ. ID NO:390]

Oligo #338 Length: 000018

AGCGAGATTT TTAAGAAT [SEQ. ID NO:391]

Oligo #339 Length: 000018

AAAAATCTCA ACCCATGT [SEQ. ID NO:392]

Oligo #340 Length: 000018

GTTGAGATTT TTAAGAAT [SEQ. ID NO:393]

Oligo #341 Length: 000018

AAAAATCTCG AACCATGT [SEQ. ID NO:394]

Oligo #342 Length: 000018

TTCGAGATTT TTAAGAAT [SEQ. ID NO:395]

Oligo #343 Length: 000018

AAAAATCTCC ACCCATGT [SEQ. ID NO:396]

Oligo #344 Length: 000018

GTGGAGATTT TTAAGAAT [SEQ. ID NO:397]

Oligo #345 Length: 000018

AAAAATCTCA TCCCATGT [SEQ. ID NO:398]

Oligo #346 Length: 000018

GATGAGATTT TTAAGAAT [SEQ. ID NO:399]

Oligo #347 Length: 000018

AAAAATCTCA TGCCATGT [SEQ. ID NO:400]

Oligo #348 Length: 000018

CATGAGATTT TTAAGAAT [SEQ. ID NO:401]

Oligo #349 Length: 000018

AAAAATCTCT TCCCATGT [SEQ. ID NO:402]

Oligo #350 Length: 000018

GAAGAGATTT TTAAGAAT [SEQ. ID NO:403]

Oligo #351 Length: 000018

AAAAATCTCT CCCCATGT [SEQ. ID NO:404]

Oligo #352 Length: 000018

GGAGAGATTT TTAAGAAT [SEQ. ID NO:405]

Oligo #353 Length: 000018

AAAAATCTCA CCCCATGT [SEQ. ID NO:406]

Oligo #354 Length: 000018

GGTGAGATTT TTAAGAAT [SEQ. ID NO:407]

Oligo #355 Length: 000018

AAAAATCTCT ACCCATGT [SEQ. ID NO:408]

Oligo #356 Length: 000018

GTAGAGATTT TTAAGAAT [SEQ. ID NO:409]

Oligo #357 Length: 000027

CTGCCCCTGG CCACGGCCGC AGCTACG [SEQ. ID NO:410]

Oligo #358 Length: 000024

ATGGATTGGA TGTCGCGTAG CTGC [SEQ. ID NO:411]

Oligo #359 Length: 000027

CTGCCCCTGG CCACGGCCGC AGGTACG [SEQ. ID NO:412]

Oligo #360 Length: 000024

ATGGATTGGA TGTCGCGTAC CTGC [SEQ. ID NO:413]

Oligo #361 Length: 000027

CTGCCCCTGG CCACGGCCGC AATCACG [SEQ. ID NO:414]

Oligo #362 Length: 000024

ATGGATTGGA TGTCGCGTGA TTGC [SEQ. ID NO:415]

Oligo #363 Length: 000021

GCTCATCCAA TCCATATCAA G [SEQ. ID NO:416]

Oligo #364 Length: 000024

ATGGATTGGA TGAGCCGTGG GTGC [SEQ. ID NO:417]

Oligo #365 Length: 000021

CAGCATCCAA TCCATATCAA G [SEQ. ID NO:418]

Oligo #366 Length: 000024

ATGGATTGGA TGCTGCGTGG GTGC [SEQ. ID NO:419]

Oligo #367 Length: 000021

CACCATCCAA TCCATATCAA G [SEQ. ID NO:420]

Oligo #368 Length: 000024

ATGGATTGGA TGGTGCGTGG GTGC [SEQ. ID NO:421]

Oligo #369 Length: 000021

AAACATCCAA TCCATATCAA G [SEQ. ID NO:422]

Oligo #370 Length: 000024

ATGGATTGGA TGTTTCGTGG GTGC [SEQ. ID NO:423]

Oligo #371 Length: 000021

CGAGCTCCAA TCCATATCAA G [SEQ. ID NO:424]

Oligo #372 Length: 000024

ATGGATTGGA GCTCGCGTGG GTGC [SEQ. ID NO:425]

Oligo #373 Length: 000021

CGAAACCCAA TCCATATCAA G [SEQ. ID NO:426]

Oligo #374 Length: 000024

ATGGATTGGG TTTCGCGTGG GTGC [SEQ. ID NO:427]

Oligo #375 Length: 000021

CGAGACCCAA TCCATATCAA G [SEQ. ID NO:428]

Oligo #376 Length: 000024

ATGGATTGGG TCTCGCGTGG GTGC [SEQ. ID NO:429]

Oligo #377 Length: 000021

CGAATCCCAA TCCATATCAA G [SEQ. ID NO:430]

Oligo #378 Length: 000024

ATGGATTGGG ATTCGCGTGG GTGC [SEQ. ID NO:431]

Oligo #379 Length: 000021

CGAAAACCAA TCCATATCAA G [SEQ. ID NO:432]

Oligo #380 Length: 000024

ATGGATTGGT TTTCGCGTGG GTGC [SEQ. ID NO:433]

Oligo #381 Length: 000021

CGAATGCCAA TCCATATCAA G [SEQ. ID NO:434]

Oligo #382 Length: 000024

ATGGATTGGC ATTCGCGTGG GTGC [SEQ. ID NO:435]

Oligo #383 Length: 000021

CGATTCCCAA TCCATATCAA G [SEQ. ID NO:436]

Oligo #384 Length: 000024

ATGGATTGGG AATCGCGTGG GTGC [SEQ. ID NO:437]

Oligo #385 Length: 000021

CGATCCCCAA TCCATATCAA G [SEQ. ID NO:438]

Oligo #386 Length: 000024

ATGGATTGGG GATCGCGTGG GTGC [SEQ. ID NO:439]

Oligo #387 Length: 000021

CGATGGCCAA TCCATATCAA G [SEQ. ID NO:440]

Oligo #388 Length: 000024

ATGGATTGGC CATCGCGTGG GTGC [SEQ. ID NO:441]

Oligo #389 Length: 000021

CGATACCCAA TCCATATCAA G [SEQ. ID NO:442]

Oligo #390 Length: 000024

ATGGATTGGG TATCGCGTGG GTGC [SEQ. ID NO:443]

Oligo #391 Length: 000034

CATCCAATCC AAATCAAGGA CGGTGACTGG AATG [SEQ. ID NO:444]

Oligo #392 Length: 000044

AATTCATTCC AGTCACCGTC CTTGATTTGG ATTGGATGTC GCGT [SEQ. ID NO:445]

Oligo #393 Length: 000034

CATCCAATCG AAATCAAGGA CGGTGACTGG AATG [SEQ. ID NO:446]

Oligo #394 Length: 000044

AATTCATTCC AGTCACCGTC CTTGATTTCG ATTGGATGTC GCGT [SEQ. ID NO:447]

Oligo #395 Length: 000034

CATCCAATCA TGATCAAGGA CGGTGACTGG AATG [SEQ. ID NO:448]

Oligo #396 Length: 000044

AATTCATTCC AGTCACCGTC CTTGATCATG ATTGGATGTC GCGT [SEQ. ID NO:449]

Oligo #397 Length: 000034

CATCCAATCT TCATCAAGGA CGGTGACTGG AATG [SEQ. ID NO:450]

Oligo #398 Length: 000044

AATTCATTCC AGTCACCGTC CTTGATGAAG ATTGGATGTC GCGT [SEQ. ID NO:451]

Oligo #399 Length: 000034

CATCCAATCT CCATCAAGGA CGGTGACTGG AATG [SEQ. ID NO:452]

Oligo #400 Length: 000044

AATTCATTCC AGTCACCGTC CTTGATGGAG ATTGGATGTC GCGT [SEQ. ID NO:453]

Oligo #401 Length: 000034

CATCCAATCg taATCAAGGA CGGTGACTGG AATG [SEQ. ID NO:454]

Oligo #402 Length: 000044

AATTCATTCC AGTCACCGTC CTTGATTACG ATTGGATGTC GCGT [SEQ. ID NO:455]

Oligo #403 Length: 000021

CGACATCCAA TCCGTATCAA G [SEQ. ID NO:456]

Oligo #404 Length: 000024

ACGGATTGGA TGTCGCGTGG GTGC [SEQ. ID NO:457]

Oligo #405 Length: 000021

CGACATCCAA TCAAAATCAA G [SEQ. ID NO:458]

Oligo #406 Length: 000024

TTTGATTGGA TGTCGCGTGG GTGC [SEQ. ID NO:459]

Oligo #407 Length: 000021

CGACATCCAA TCTACATCAA G [SEQ. ID NO:460]

Oligo #408 Length: 000024

GTAGATTGGA TGTCGCGTGG GTGC [SEQ. ID NO:461]

Oligo #409 Length: 000016

GCTGGTGACT GGAATG [SEQ. ID NO:462]

Oligo #410 Length: 000026 [SEQ. ID NO:463]

AATTCATTCC AGTCACCAGC CTTGAT

Oligo #411 Length: 000016

AACGGTGACT GGAATG [SEQ. ID NO:464]

Oligo #412 Length: 000026

AATTCATTCC AGTCACCGTT CTTGAT [SEQ. ID NO:465]

Oligo #413 Length: 000016

GAAGGTGACT GGAATG [SEQ. ID NO:466]

Oligo #414 Length: 000026

AATTCATTCC AGTCACCTTC CTTGAT [SEQ. ID NO:467]

Oligo #415 Length: 000016

GGTGGTGACT GGAATG [SEQ. ID NO:468]

Oligo #416 Length: 000026

AATTCATTCC AGTCACCACC CTTGAT [SEQ. ID NO:469]

Oligo #417 Length: 000016

ATCGGTGACT GGAATG [SEQ. ID NO:470]

Oligo #418 Length: 000026

AATTCATTCC AGTCACCGAT CTTGAT [SEQ. ID NO:471]

Oligo #419 Length: 000016

CTGGGTGACT GGAATG [SEQ. ID NO:472]

Oligo #420 Length: 000026

AATTCATTCC AGTCACCCAG CTTGAT [SEQ. ID NO:473]

Oligo #421 Length: 000016

TTCGGTGACT GGAATG [SEQ. ID NO:474]

Oligo #422 Length: 000026

AATTCATTCC AGTCACCGAA CTTGAT [SEQ. ID NO:475]

Oligo #423 Length: 000016

TCCGGTGACT GGAATG [SEQ. ID NO:476]

Oligo #424 Length: 000026

AATTCATTCC AGTCACCGGA CTTGAT [SEQ. ID NO:477]

Oligo #425 Length: 000032

AATTCGCTAG GAAACTGACG TTCTATCTGA AA [SEQ. ID NO:478]

Oligo #426 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGTTT CCTAGCG [SEQ. ID NO:479]

Oligo #427 Length: 000032

AATTCCAGAG GAAACTGACG TTCTATCTGA AA [SEQ. ID NO:480]

Oligo #428 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGTTT CCTCTGG [SEQ. ID NO:481]

Oligo #429 Length: 000032

AATTCCACAG GAAACTGACG TTCTATCTGA AA [SEQ. ID NO:482]

Oligo #430 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGTTT CCTGTGG [SEQ. ID NO:483]

Oligo #431 Length: 000032

AATTCTCCAG GAAACTGACG TTCTATCTGA AA [SEQ. ID NO:484]

Oligo #432 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGTTT CCTGGAG [SEQ. ID NO:485]

Oligo #433 Length: 000032

AATTCCGGAG GCGTCTGACG TTCTATCTGA AA [SEQ. ID NO:486]

Oligo #434 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGACG CCTCCGG [SEQ. ID NO:487]

Oligo #435 Length: 000032

AATTCCGGAG GGAACTGACG TTCTATCTGA AA [SEQ. ID NO:488]

Oligo #436 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGTTC CCTCCGG [SEQ. ID NO:489]

Oligo #437 Length: 000032

AATTCCGGAG GCACCTGACG TTCTATCTGA AA [SEQ. ID NO:490]

Oligo #438 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGGTG CCTCCGG [SEQ. ID NO:491]

Oligo #439 Length: 000032

AATTCCGGAG GATCCTGACG TTCTATCTGA AA [SEQ. ID NO:492]

Oligo #440 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGGAT CCTCCGG [SEQ. ID NO:493]

Oligo #441 Length: 000032

AATTCCGGAG GTCCCTGACG TTCTATCTGA AA [SEQ. ID NO:494]

Oligo #442 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGGGA CCTCCGG [SEQ. ID NO:495]

Oligo #443 Length: 000032

AATTCCGGAG GAAACTGACG GACTATCTGA AA [SEQ. ID NO:496]

Oligo #444 Length: 000037

CTCAAGGGTT TTCAGATAGT CCGTCAGTTT CCTCCGG [SEQ. ID NO:497]

Oligo #445 Length: 000032

AATTCCGGAG GAAACTGACG ATCTATCTGA AA [SEQ. ID NO:498]

Oligo #446 Length: 000037

CTCAAGGGTT TTCAGATAGA TCGTCAGTTT CCTCCGG [SEQ. ID NO:499]

Oligo #447 Length: 000032

AATTCCGGAG GAAACTGACG CTGTATCTGA AA [SEQ. ID NO:500]

Oligo #448 Length: 000037

CTCAAGGGTT TTCAGATACA GCGTCAGTTT CCTCCGG [SEQ. ID NO:501]

Oligo #449 Length: 000032

AATTCCGGAG GAAACTGACG AAATATCTGA AA [SEQ. ID NO:502]

Oligo #450 Length: 000037

CTCAAGGGTT TTCAGATATT TCGTCAGTTT CCTCCGG [SEQ. ID NO:503]

Oligo #451 Length: 000032

AATTCCGGAG GAAACTGACG GTTTATCTGA AA [SEQ. ID NO:504]

Oligo #452 Length: 000037

CTCAAGGGTT TTCAGATAAA CCGTCAGTTT CCTCCGG [SEQ. ID NO:505]

Oligo #453 Length: 000032

AATTCCGGAG GAAACTGACG TTCTATCTGG CT [SEQ. ID NO:506]

Oligo #454 Length: 000037

CTCAAGGGTA GCCAGATAGA ACGTCAGTTT CCTCCGG [SEQ. ID NO:507]

Oligo #455 Length: 000032

AATTCCGGAG GAAACTGACG TTCTATCTGC GT [SEQ. ID NO:508]

Oligo #456 Length: 000037

CTCAAGGGTA CGCAGATAGA ACGTCAGTTT CCTCCGG [SEQ. ID NO:509]

Oligo #457 Length: 000032

AATTCCGGAG GAAACTGACG TTCTATCTGA AC [SEQ. ID NO:510]

Oligo #458 Length: 000037

CTCAAGGGTG TTCAGATAGA ACGTCAGTTT CCTCCGG [SEQ. ID NO:511]

Oligo #459 Length: 000032

AATTCCGGAG GAAACTGACG TTCTATCTGC AG [SEQ. ID NO:512]

Oligo #460 Length: 000037

CTCAAGGGTC TGCAGATAGA ACGTCAGTTT CCTCCGG [SEQ. ID NO:513]

Oligo #461 Length: 000032

AATTCCGGAG GAAACTGACG TTCTATCTGC AC [SEQ. ID NO:514]

Oligo #462 Length: 000037

CTCAAGGGTG TGCAGATAGA ACGTCAGTTT CCTCCGG [SEQ. ID NO:515]

Oligo #463 Length: 000032

AATTCCGGAG GAAACTGACG TTCTATCTGA TG [SEQ. ID NO:516]

Oligo #464 Length: 000037

CTCAAGGGTC ATCAGATAGA ACGTCAGTTT CCTCCGG [SEQ. ID NO:517]

Oligo #465 Length: 000032

AATTCCGGAG GAAACTGACG TTCTATCTGT TC [SEQ. ID NO:518]

Oligo #466 Length: 000037

CTCAAGGGTG AACAGATAGA ACGTCAGTTT CCTCCGG [SEQ. ID NO:519]

Oligo #467 Length: 000032

AATTCCGGAG GAAACTGACG TTCTATCTGT AC [SEQ. ID NO:520]

Oligo #468 Length: 000037

CTCAAGGGTG TACAGATAGA ACGTCAGTTT CCTCCGG [SEQ. ID NO:521]

Oligo #469 Length: 000040

CATGGCTAAC TGCTCTAACA TGATCGATGA AATTATAACA [SEQ. ID NO:522]

Oligo #470 Length: 000036

CACTTAAAGC AGCCACCTTT GCCTTTGCTG GACTTC [SEQ. ID NO:523]

Oligo #471 Length: 000027

AACAACCTCA ATGGGGAAGA CCAAGAT [SEQ. ID NO:524]

Oligo #472 Length: 000045

CTTTAAGTGT GTTATAATTT CATCGATCAT GTTAGAGCAG TTAGC [SEQ. ID NO:525]

Oligo #473 Length: 000036

GAGGTTGTTG AAGTCCAGCA AAGGCAAAGG TGGCTG [SEQ. ID NO:526]

Oligo #474 Length: 000018

ATCTTGGTCT TCCCCATT [SEQ. ID NO:527]

Oligo #475 Length: 000036

ATCCTGATGG AAAATAACCT TCGAAGGCCA AACCTG [SEQ. ID NO:528]

Oligo #476 Length: 000024

GAGGCATTCA ACAGGGCTGT CAAG [SEQ. ID NO:529]

Oligo #477 Length: 000015

AGTTTACAGA ATGCA [SEQ. ID NO:530]

Oligo #478 Length: 000027

CCTTCGAAGG TTATTTTCCA TCAGGAT [SEQ. ID NO:531]

Oligo #479 Length: 000024

CCTGTTGAAT GCCTCCAGGT TTGG [SEQ. ID NO:532]

Oligo #480 Length: 000020

TTCTGTAAAC TCTTGACAGC [SEQ. ID NO:533]

Oligo #481 Length: 000021

TCAGCAATTG AGAGCATTCT T [SEQ. ID NO:534]

Oligo #482 Length: 000018

AAAAATCTCC TGCCATGT [SEQ. ID NO:535]

Oligo #483 Length: 000048

CTGCCCCTGG CCACGGCCGC ACCCACGCGA CATCCAATCC ATATCAAG [SEQ. ID NO:536]

Oligo #484 Length: 000027

CTGCCCCTGG CCACGGCCGC ACCCACG [SEQ. ID NO:537]

Oligo #485 Length: 000021

CGACATCCAA TCCATATCAA G [SEQ. ID NO:538]

Oligo #486 Length: 000016

GACGGTGACT GGAATG [SEQ. ID NO:539]

Oligo #487 Length: 000019

GCTCTCAATT GCTGATGCA [SEQ. ID NO:540]

Oligo #488 Length: 000018

CAGGAGATTT TTAAGAAT [SEQ. ID NO:541]

Oligo #489 Length: 000048

ATGGATTGGA TGTCGCGTGG GTGCGGCCGT GGCCAGGGGC AGACATGG [SEQ. ID NO:542]

Oligo #490 Length: 000024

GGCCGTGGCC AGGGGCAGAC ATGG [SEQ. ID NO:543]

Oligo #491 Length: 000024

ATGGATTGGA TGTCGCGTGG GTGC [SEQ. ID NO:544]

Oligo #492 Length: 000026

AATTCATTCC AGTCACCGTC CTTGAT [SEQ. ID NO:545]

Oligo #493 Length: 000032

AATTCCGGAG GAAACTGACG TTCTATCTGA AA [SEQ. ID NO:546]

Oligo #494 Length: 000032

ACCCTTGAGA ATGCGCAGGC TCAACAGTAA TA [SEQ. ID NO:547]

Oligo #495 Length: 000037

CTCAAGGGTT TTCAGATAGA ACGTCAGTTT CCTCCGG [SEQ. ID NO:548]

Oligo #496 Length: 000027

AGCTTATTAC TGTTGAGCCT GCGCATT [SEQ. ID NO:549]

TABLE 3

POLYPEPTIDES

PEPTIDE #1; pMON5988 (Example 9); (15-125)hIL-3

```
Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15                  20                  25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
30                  35                  40

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
45                  50                  55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60                  65                  70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75                  80                  85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90                  95                  100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
105                 110                 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:65]
120                 125
```

PEPTIDE #A1; pMON13304 (Example 55); Met-Ala-(15-125)hIL-3 (98I, 100R);

```
Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15                  20                  25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
30                  35                  40

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
45                  50                  55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60                  65                  70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75                  80                  85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile Ile Ile Arg Asp Gly
90                  95                  100
```

```
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
        105                 110                 115

Leu Glu Asn Ala Gln Ala Gln Gln  [SEQ ID NO:66]
        120                 125


PEPTIDE #A2; pMON13305 Met-Ala-(15-125)hIL-3; (95R, 98I, 100R);

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
        15                  20                  25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
        30                  35                  40

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
        45                  50                  55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
        60                  65                  70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
        75                  80                  85

Ala Thr Ala Ala Pro Thr Arg Arg Pro Ile Ile Ile Arg Asp Gly
        90                  95                  100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
        105                 110                 115

Leu Glu Asn Ala Gln Ala Gln Gln  [SEQ ID NO:67]
        120                 125


PEPTIDE #A3; pMON13286 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S);

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
        15                  20                  25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Asp
        30                  35                  40

Glu Asp Met Ser Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
        45                  50                  55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
        60                  65                  70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
        75                  80                  85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
        90                  95                  100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
        105                 110                 115
```

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:69]
120 125

Polypeptides corresponding to SEQ ID NOS. 15, 16, 17, 18 and 129 comprising (1-133)hIL-3 containing one or more amino acid substitutions can be made using the procedures described above and in the following examples by starting with the appropriate oligonuctiotides and then constructing the DNA encoding the polypeptide and expressing it in an appropriate host cell. In a similar manner polypeptides which correspond to SEQ ID NOS. 19, 20, 21, 22 and 130 and contain one or more amino acid substitutions and wherein from 1 to 14 amino acids have been sequentially deleted from the N-terminus, or from 1 to 15 amino acids have been deleted from the C-terminus or deletions of amino acids have been made from both the N-terminus and the C-terminus can also be made by following the procedures described above and in the following examples, beginning with the appropriate starting materials.

Additional details may be found in United States Patent Application Serial No. 07/981,044 filed November 24, 1992, which is hereby incorporated by reference in its entirety.

Additional details may be found in co filed United States Patent Application Attorney docket number 2713/2, which is hereby incorporated by reference in its entirety.

All references, patents or applications cited herein are incorporated by reference in their entirety.

Further details known to those skilled in the art may be found in T. Maniatis, et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory (1982) and references cited therein, incorporated herein by reference in its entirety; and in J. Sambrook, et al., Molecular Cloning, A Laboratory Manual, 2nd edition, Cold

Spring Harbor Laboratory (1989) and references cited therein, incorporated herein by reference in its entirety.

The following examples will illustrate the invention in greater detail although it will be understood that the invention is not limited to these specific examples.

Amino acids are shown herein by standard one letter or three letter abbreviations as follows:

Abbreviated Designation		Amino Acid
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

Various other examples will be apparent to the person skilled in the art after reading the present disclosure without departing from the spirit and scope of the invention. It is intended that all such other

examples be included within the scope of the appended claims.

References

Adams, S.P., Kavka, K.S., Wykes, E.J., Holder, S.B. and Galluppi, G.R. Hindered Dialkyamino Nucleoside Phosphate reagents in the synthesis of two DNA 51-mers. J. Am. Chem. Soc., 105, 661-663 (1983).

Atkinson, T. and Smith, M., in Gait, M.J., Oligonucleotide Sythesis (1984) (IRL Press, Oxford England).

Bachmann, B., Pedigrees of some mutant strains of Escherichia coli K-12, Bacteriological Reviews, 36:525-557 (1972).

Bayne, M. L., Expression of a synthetic gene encoding human insulin-like growth factor I in cultured mouse fibroblasts. Proc. Natl. Acad. Sci. USA 84, 2638-2642 (1987).

Ben-Bassat, A., K. Bauer, S-Y. Chang, K. Myambo, A. Boosman and S. Ching. Processing of the initiating methionine from proteins: properties of the Escherichia coli methionine aminopeptidase and its gene structure. J. Bacteriol., 169: 751-757 (1987).

Biesma, B. et al., Effects of interleukin-3 after chemotherapy for advanced ovarian cancer. Blood, 80:1141-1148 (1992).

Birnboim, H. C. and J. Doly. A rapid alkaline extraction method for screening recombinant plasmid DNA. Nucleic Acids Research, 7(6): 1513-1523 (1979).

Bradford, M. M., A rapid and sensitive method for the

quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Analytical Biochemistry, 72: 248-254 (1976).

Clark-Lewis, I., L. E. Hood and S. B. H. Kent. Role of disulfide bridges in determining the biological activity of interleukin 3, Proc. Natl. Acad. Sci.,USA, 85: 7897-7901 (1988).

Clement, J. M. and Hofnung, M. Gene sequence of the receptor, an outer membrane protein of E. coli K12. Cell, 27: 507-514 (1981).

Covarrubias, L., L. Cervantes, A. Covarrubias, X. Soberon, I. Vichido, A. Blanco, Y. M. Kupersztoch-Portnoy and F. Bolivar. Construction and characterization of new cloning vehicles. V. Mobilization and coding properties of pBR322 and several deletion derivates including pBR327 and pBR328. Gene 13: 25-35 (1981).

Deng, W.P. & Nickoloff, J.A. Site-directed mutagenesis of virtually any plasmid by eliminating a unique site. Anal. Biochem. 200:81 (1992).

Dente, L., G. Cesareni and R. Cortese, pEMBL: A new family of single stranded plasmids, Nucleic Acids Research, 11: 1645-1655 (1983).

Dunn, J.J. and Studier, F.W., Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. J. Mol. Biol. 166:477-535 (1983).

Falk, S., G. Seipelt, A. Ganser, O. G. Ottmann, D. Hoelzer, H. J. Stutte and K. Hubner. Hematopathology 95: 355 (1991).

Fling, M. E., et al. Nucleotide sequence of the

transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase. Nucl. Acids Res. 13:7095-7106 (1985).

Ganser, A., A. Lindemann, G. Seipelt, O. G. Ottmann, F. Herrmann, M. Eder, J. Frisch, G. Schulz, R. Mertelsmann and D. Hoelzer. Effects of Recombinant Human Interleukin-3 in Patients With Normal Hematopoiesis and in Patients with Bone Marrow Failure, Blood 76: 666 (1990).

Gething and Sambrook, Cell-surface expression of influenza haemagglutinin from a cloned DNA copy of the RNA gene, Nature, 293: 620-625 (1981).

Gillio, A. P., C. Gasparetto, J. Laver, M. Abboud, M. A. Bonilla, M. B. Garnick and R. J. O'Reilly. J. Clin. Invest. 85: 1560 (1990).

Gouy, M. and G. Gautier, Codon usage in bacteria: Correlation with gene expressivity, Nucleic Acids Research, 10: 7055-7074 (1982).

Greenfield, L., T. Boone, and G. Wilcox. DNA sequence of the araBAD promoter in Escherichia coli B/r. Proc. Natl. Acad. Sci. USA, 75: 4724-4728 (1978).

Higuchi, R, (1989) in *PCR Technology*, H.A. Erlich ed., Stockton Press, N.Y. chapter 2-6.

Hunkapiller, M. W., R. W. Hewick, R. J. Dreyer and L. E. Hood. High sensitivity sequencing with a gas-phase sequenator. Methods in Enzymology 153: 399-413 (1983).

Kaufman, et al., Coamplification and Coexpression of Human Tissue-Type Plasminogen Activator and Murine Dihydrofolate Reductase Sequences in Chinese Hamster Ovary Cells, Mol. Cell. Biol., 5(7): 1750-1759 (1985).

Kaufman, R. J. High level production of proteins in mammalian cells, in Genetic Engineering, Principles and Methods, Vol. 9, J. K. Setlow, editor, Plenum Press, New York (1987).

Kunkel, T. A. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA, 82: 488-492 (1985).

Laemmli, U. K., Cleavage of structural proteins during assembly of the head of bacteriophage T4, Nature, 227:680-685 (1970).

Lange, B., M. Valtieri, D. Santoli, D. Caracciolo, F. Mavilio, I. Gemperlein, C. Griffin, B. Emanuel, J. Finan, P. Nowell, and G. Rovera. Growth factor requirements of childhood acute leukemia: establishment of GM-CSF-defendent cell lines. Blood 70:192 (1987).

Mahler, H. R. and E. H. Cordes, in Biological Chemistry, p. 128, New York, Harper and Row (1966).

Maniatis, T., E. F. Fritsch and J. Sambrook, Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory (1982).

Marinus, M. G. Location of DNA methylation genes on the Escherichia coli K-12 genetic map. Molec. Gen. Genet. 127: 47-55 (1973).

McBride, L.J. and Caruthers, M.H. An investigation of several deoxynucleoside phosphoramidites. Tetrahedron Lett., 24, 245-248 (1983).

Messing, J., A multipurpose cloning system based on the single-stranded DNA bacteriophage M13. Recombinant DNA Technical Bulletin, NIH Publication No. 79-99, Vol. 2,

No. 2, pp. 43-48 (1979).

Neu, H. C. and L. A. Heppel. The release of enzymes from Escherichia coli by osmotic shock and during the formation of spheroplasts. J. Biol. Chem., 240: 3685-3692 (1965).

Obukowicz, M.G., Staten, N.R. and Krivi, G.G., Enhanced Heterologous Gene Expression in Novel rpoH Mutants of Escherichia coli. Applied and Environmental Microbiology 58, No. 5, p. 1511-1523 (1992).

Olins, P. O., C. S. Devine, S. H. Rangwala and K. S. Kavka, The T7 phage gene 10 leader RNA, a ribosome-binding site that dramatically enhances the expression of foreign genes in Escherichia coli, Gene, 73:227-235 (1988).

Olins, P. O. and S. H. Rangwala, Vector for enhanced translation of foreign genes in Escherichia coli, Methods in Enzymology, 185: 115-119 (1990).

Postmus, et al., Effects of recombinant human interleukin-3 in patients with relapsed small-cell lung cancer treated with chemotherapy: a dose-finding study. J. Clin. Oncol., 10:1131-1140 (1992).

Prober, J. M., G. L. Trainor, R. J. Dam, F. W. Hobbs, C. W. Robertson, R. J. Zagursky, A. J. Cocuzza, M. A. Jensen and K. Baumeister. A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides. Science 238: 336-341 (1987).

Renart J., J. Reiser and G. R. Stark, Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with anti-sera: a method for studying antibody specificity and antigen structure, Proc. Natl. Acad. Sci. USA, 76:3116-3120 (1979).

Saiki, R.K., Schorf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A. and Arnheim, N., Enzymatic Amplification of ß-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia, Science, 230: 1350-1354 (1985).

Sambrook, J., et al., Molecular Cloning, A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory (1989).

Sancar, A., C. Stachelek, W. Konigsberg and W. D. Rupp, Sequences of the recA gene and protein, Proc. Natl. Acad. Sci., 77: 2611-2615 (1980).

Sanger, F., S. Nicklen and A. R. Coulson. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U. S. A. 74: 5463-5467 (1977).

Santoli, D., Y. Yang, S. C. Clark, B. L. Kreider, D. Caracciolo, and G. Rovera. Synergistic and antagonistic effects of recombinant human interleukin (IL-3), IL-1α, granulocyte and macrophage colony-stimulating factors (G-CSF and M-CSF) on the growth of GM-CSF-dependent leukemic cell lines. J. Immunol. 139:348 (1987).

Smith, M. In vitro mutagenesis. Ann. Rev. Genet., 19:423-462 (1985).

Soberon, X., L. Covarrubias and F. Bolivar, Construction and characterization of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325, Gene, 9: 211-223 (1980).

Stader, J. A. and T. J. Silhavy. Engineering Escherichia coli to secrete heterologous gene products, Methods in Enzymology, 185: 166-87 (1990).

Summers, M. D. and G. E. Smith. A manual of methods for

Baculovirus vectors and insect cell culture procedures. Texas Agricultural Experiment Station Bulletin No. 1555 (1987).

Taylor, J.W., Ott, J. and Eckstein, F.. The rapid generation of oligonucleotide-directed mutants at high frequency using phosphorothioate modified DNA. Nucl. Acids Res., 13:8764-8785 (1985).

Treco, D.A., in *Current protocols in Molecular Biology*, Seidman et al., eds. J Wiley N.Y., unit 2.1. (1989)

Valtieri, M., D. Santoli, D. Caracciolo, B. L. Kreider, S. W. Altmann, D. J. Tweardy, I. Gemperlein, F. Mavilio, B. J. Lange and G. Rovera. Establishment and characterization of an undifferentiated human T leukemia cell line which requires granulocyte-macrophage colony stimulating factor for growth. J. Immunol. 138:4042 (1987).

Voet, D., W. B. Gatzer, R. A. Cox, P. Doty. Absorption spectra of the common bases. Biopolymers 1: 193 (1963).

Wells, J.A., Vasser, M., and Powers, D.B. Cassette mutagenesis: an effective method for generation of multiple mutants at defined sites. Gene, 34:315-323 (1985).

Wong, E. Y., R. Seetharam, C. Kotts, R. A. Heeren, B. K. Klein, S. B. Braford, K. J. Mathis, B. F. Bishop, N. R. Siegel, C. E. Smith and W. C. Tacon. Expression of secreted IGF-1 in Escherichia coli. Gene, 68: 193-203 (1988).

Yang, Y., A. B. Clarletta, P.A. Temple, M.P. Chung, S. Koviacic, J. S. Witek-Giannotti, A.C. Leary, R. Kriz, R.E. Donahue, G.G. Wong and S.C. Clark. Human IL-3 (Multi-CSF): Identification by expression cloning of a novel hematopoietic growth factor related to murine IL-3. Cell 47: 3-10 (1985).

Yanisch-Perron, C., J. Viera and J. Messing. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33: 103-119 (1985).

Zoller, M.J. and Smith, M. Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA. Nucleic Acid Research, 10: 6487-6500 (1982).

Zoller, M.J. and Smith, M. Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors. Methods in Enzymology, 100:468-500 (1983).

Zoller, M.J. and Smith, M. Oligonucleotide-directed Mutagenesis: A simple method using two oligonucleotide primers and a single-stranded DNA template. DNA, 3: 479 (1984).

EXAMPLE 1

Construction of pMON 5846 (Fig. 4) which encodes [Met-(1-133)hIL-3 (Arg^{129})]

A plasmid containing the gene for the cDNA of hIL-3 cloned into pUC18 on an EcoRI to HindIII fragment was obtained from British Biotechnology Limited (Cambridge, England). This plasmid was designated pPO518. The purified plasmid DNA was cleaved by the restriction endonucleases NheI and BamHI. Approximately 0.5 micrograms of cleaved plasmid DNA was ligated to 1.0 picomoles of a pair of annealed oligonucleotides with the following sequence:

5'-CTAGCGATCTTTTAATAAGCTTG-3' [SEQ ID NO: 1]
3'-GCTAGAAAATTATTCGAACCTAG-5' [SEQ ID NO: 2]

The ligation mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked, and plasmid DNA was purified and subjected to restriction enzyme analysis. An isolate was identified in which the above oligonucleotide sequence had replaced the portion of the gene that encodes the extreme C-terminus. Within the new sequence was a new stop codon, TAA, and a recognition site for the enzyme HindIII. The new plasmid was designated pMON5846.

EXAMPLE 2

(a) Construction of expression vector plasmid pMON2341

The plasmid pMON2341 was used to supply the

particular replicon and expression elements used for construction of many of the plasmids used to produce hIL-3 and hIL-3 muteins in E. coli. These expression elements are described in the materials and methods section. pMON2341 is derived from pMON5515 (Olins et al., 1988) and from pMON2429. pMON2429 consists of the phage mp18 (Yanisch-Perron et al., 1985) with a BclI fragment carrying the chloramphenicol acetyl transferase (cat) gene from pBR328 (Covarrubias et al., 1981) inserted into the BamHI site. The cat gene in pMON2429 has been altered from that in pBR328 by site directed mutagenesis (Kunkel, 1985). The recognition sites for NcoI and EcoRI which occur in the native gene were altered so that these two restriction enzymes no longer recognize these sites. The changes did not alter the protein specified by the gene. Also, an NcoI site was introduced at the N-terminus of the coding sequence so that it overlaps the codon for initiator methionine.

The steps involved in construction of pMON2341 are listed below:

(1) The DNAs of pMON5515 and pMON2429 were treated with NcoI and HindIII. The fragments were ligated and used to transform competent E. coli to ampicillin resistance. From these colonies, some were identified that were chloramphenicol resistant. From one of these colonies, plasmid DNA was isolated in which the rat atriopeptigen gene of pMON5515 had been replaced by the NcoI to HindIII fragment containing the cat gene from pMON2429. This fragment contains the recognition sites for several restriction enzymes in the portion derived from the multilinker region of mp18. The new plasmid was designated pMON2412.

(2) pMON2412 was treated with the enzyme ClaI which cleaves at one location in the pBR327 derived portion of the DNA. The protruding ends were rendered blunt by

treatment with Klenow in the presence of nucleotide precursors. This DNA was mixed with an isolated 514 bp RsaI fragment derived from pEMBL8 (Dente et al., 1983). This RsaI fragment contains the origin of replication of phage f1. This ligation mixture was used to transform competent E. coli cells to ampicillin resistance. Among the plasmid DNAs isolated from these cells was pMON5578. This plasmid has the structure of pMON2412 with the f1 origin region inserted into the ClaI site. This is illustrated in the Figures and in Olins and Rangwala (1990).

(3) The DNA of pMON5578 was treated with restriction enzymes HindIII and MstII. The DNA was then treated with Klenow enzyme in the presence of nucleotide precursors to render the ends blunt. This treated DNA was ligated and used to transform competent E. coli to ampicillin resistance. From the ampicillin resistant colonies, one plasmid was recovered from which the portion between HindIII and MstII was absent. This deletion resulted in the removal of sequences from the plasmid which are recognized by a number of restriction endonuclease sites. The new plasmid was designated pMON5582.

(4) The DNA of pMON5582 was treated with SstII and BclI and ligated in the presence of annealed oligonucleotides with the sequences shown below.

5'- GGCAACAATTTCTACAAAACACTTGATACTGTATGAGCAT-
3'-CGCCGTTGTTAAAGATGTTTTGTGAACTATGACATACTCGTA-

ACAGTATAATTGCTTCAACAGAACAGATC-3' [SEQ ID NO:3]
TGTCATATTAACGAAGTTGTCTTGT-5' [SEQ ID NO:4]

This sequence encodes the essential elements of the recA promoter of E. coli including the transcription start site and the lexA repressor binding site (the operator) (Sancar et al., 1980). The plasmid recovered

from the ligation mixes contained this recA promoter in place of the one in pMON5582 (and in pMON5515). The functionality of the recA promoter was illustrated by Olins and Rangwala (1990). The new plasmid was designated pMON5594.

(5) To eliminate the single EcoRI site in pMON5594, the DNA was treated with EcoRI, then with Klenow in the presence of nucleotide precursors to render the ends blunt and then the DNA was ligated. From this ligation mix a plasmid was recovered whose DNA was not cleaved with EcoRI. This plasmid was designated pMON5630.

(6) To alter the single recognition site for PstI, plasmid pMON5630 was subjected to site directed mutagenesis (Kunkel, 1985). The oligonucleotide used in this procedure has the sequence shown below.

5'-CCATTGCTGCCGGCATCGTGGTC-3' [SEQ ID NO:5]

The result of the procedure was to construct pMON2341 which differs from pMON5630 in that the PstI site in the beta-lactamase gene was altered so that PstI no longer recognizes the site. The single nucleotide change does not alter the amino acid sequence of the beta-lactamase protein.

(b) Construction of pMON5847 (Fig. 5) which encodes [Met-(1-133)hIL-3(Arg^{129})]

Plasmid pMON2341 was used to supply the replicon, promotor, ribosome binding site, transcription terminator and antibiotic resistance marker for the plasmids used to produce hIL-3 in E. coli from cDNA derived hIL-3 genes.

Plasmid pMON2341 was treated with restriction enzymes NcoI and HindIII. The restriction fragment containing the replication origin was purified. The DNA

of plasmid pMON5846 was treated with NcoI and HindIII. The restriction fragment containing the hIL-3 gene was gel purified. These purified restriction fragments were mixed and ligated. The ligation mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked, and plasmid DNA was purified and analyzed using restriction enzymes. pMON5847 was identified as a plasmid with the replicon of pMON2341 and the hIL-3 gene in place of the chloramphenicol acetyl transferase gene. JM101 cells harboring this plasmid were cultured in M9 medium and treated with nalidixic acid as described above. Samples of the culture were examined for protein content. It was found that this hIL-3 mutein was produced at about 6% of total cell protein as measured on Coomassie stained polyacrylamide gels.

EXAMPLE 3

Construction of pMON5854 (Fig. 7) which encodes [Met-(1-133)hIL-3(Arg^{129})]

To increase the accumulation of hIL-3 in E. coli, the coding sequence of the amino terminal portion of the protein was altered to more closely reflect the codon bias found in E. coli genes that produce high levels of proteins (Gouy and Gautier, 1982). To change the coding sequence for the amino terminal portion of the gene, a pair of synthetic oligonucleotides were inserted between the NcoI and HpaI sites within the coding sequence. About 0.5 micrograms of DNA of the plasmid pMON5847 (Example 2) was treated with NcoI and HpaI. This DNA was mixed with an annealed pair of oligonucleotides with the following sequence:

5'-CATGGCTCCAATGACTCAGACTACTTCTCTTAAGACT-
3'-CGAGGTTACTGAGTCTGATGAAGAGAATTCTGA-

TCTTGGGTT-3' [SEQ ID NO:6]
AGAACCCAA-5' [SEQ ID NO:7]

The fragments were ligated. The ligation mixture was used to transform competent JM101 to ampicillin resistance. Colonies were picked into broth. From the cultures plasmid DNA was made and examined for the presence of a DdeI site (CTNAG) which occurs in the synthetic sequence but not between the NcoI and HpaI sites in the sequence of pMON5847. The new recombinant plasmid was designated pMON5854. The nucleotide sequence of the DNA in the coding sequence of the amino terminal portion of the hIL-3 gene in pMON5854 was determined by DNA sequencing and found to be the same as that of the synthetic oligonucleotide used in ligation. Cultures of JM101 cells harboring this plasmid were grown and treated with nalidixic acid to induce production of the hIL-3 mutant protein. Analysis of the proteins on Coomassie gels showed that the accumulation of hIL-3 mutein was about 25% of total cell protein in cultures harboring pMON5854, significantly higher than it was in cultures harboring pMON5847.

EXAMPLE 4

Construction of pMON5887 (Fig. 12) which encodes [Met-(1-125)hIL-3]

The plasmid DNA of pMON5854 (Example 3) was treated with EcoRI and HindIII and the larger fragment was gel purified. About 0.5 microgram of this DNA was ligated to 1 picomole of an annealed pair of oligonucleotides which encode amino acids 107 through 125 of hIL-3. The sequences of these oligonucleotides are shown below.

EcoRI to HindIII

5'-AATTCCGTCGTAAACTGACCTTCTATCTGAAAA-
3'-GGCAGCATTTGACTGGAAGATAGACTTTT-

CCTTGGAGAACGCGCAGGCTCAACAGTAATA-3' [SEQ ID NO:8]
GGAACCTCTTGCGCGTCCGAGTTGTCATTATTCGA-5' [SEQ ID NO:9]

After ligation, the DNA was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked into broth and plasmid DNA was isolated from each culture. Restriction analysis of the plasmid DNA showed the presence of an EcoRI to HindIII fragment smaller than that of pMON5854. The nucleotide sequence of the portion of the coding sequence between the EcoRI and HindIII sites was determined to confirm the accuracy of the replaced sequence. The new plasmid was designated pMON5887 encoding Met-(1-125)hIL-3 which has the following amino acid sequence:

Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser
Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
Thr Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:10]

EXAMPLE 5

Construction of pMON5967 which encodes [Met-Ala-(15-125)hIL-3]

Plasmid DNA of pMON5887 isolated from E. coli GM48 (dam-) was cleaved with NcoI and ClaI and ligated to 1 picomole of an annealed pair of oligonucleotides, Nco I and ClaI, encoding amino acids [Met Ala (15-20)hIL-3]. The sequence of these oligonucleotides is shown below.

5'-CATGGCTAACTGCTCTAACATGAT-3'[SEQ ID NO:11]

3'-CGATTGACGAGATTGTACTAGC-5'[SEQ ID NO:12]

The resulting ligation mix was used to transform competent E. coli JM101 cells to ampicillin resistant colonies. Plasmid DNA was isolated from these cells and the size of the inserted fragment was determined to be smaller than that of pMON5887 by restriction analysis using NcoI and NsiI. The nucleotide sequence of the region between NcoI and ClaI was determined and found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5967 and cells containing it were induced for protein production. Sonicated cell pellets and supernatants were used for protein purification and bio-assay.

EXAMPLE 6

Construction of pMON5978 which encodes [Met-Ala-(15-125)hIL-3]

Plasmid DNA of pMON5967 isolated from E. coli GM48(dam-) was cleaved with ClaI and NsiI and ligated to 1 picomole of an annealed assembly of six oligonucleotides encoding hIL-3 amino acids 20-70 (FIG. 2). This synthetic fragment encodes three unique restriction sites, EcoRV, XhoI and PstI. The sequence of these oligonucleotides is shown in Figure 2.

The resulting ligation mix was used to transform competent E. coli JM101 cells to ampicillin resistant colonies. Plasmid DNA was isolated and screened with XbaI and EcoRV for the presence of the new restriction site EcoRV. The DNA sequence of the region between ClaI and NsiI was determined and found to be the same as that of the synthetic oligonucleotides. The new plasmid was designated pMON5978, and cells containing it were induced for protein production. Sonicated cell pellets and supernatants were used for protein purification and bio-

assay.

Plasmid pMON5978 encodes [Met-Ala-(15-125)hIL-3] which has the following amino acid sequence:

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
Thr Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:13]

EXAMPLE 7

Construction of pMON5898

Plasmid pMON5851 DNA was digested with restriction enzymes *HindIII* and *NcoI* resulting in a 3695 base pair NcoI,HindIII fragment. The genetic elements derived from pMON5851 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, *AraBAD* promoter, g10L ribosome binding site and the *lam*B secretion leader. The AraBAD promoter is identical to that described in plasmid pMON6235 and the lamB signal peptide sequence used is that shown in Figure 8 fused to hIL-3 at the NcoI recognition site. Plasmid pMON5873 DNA was digested with restriction enzymes *HindIII* and *NcoI* resulting in a 408 base pair NcoI,HindIII fragment. The genetic element derived from pMON5873 is the hIL-3 gene (1-133). Clones containing the hIL-3 (1-133) gene contained a 408 base pair *NcoI*, *HindIII* restriction fragment. This construct was designated pMON5898.

EXAMPLE 8

Construction of pMON5987

Plasmid pMON6458 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3940 base pair NcoI,HindIII fragment. The genetic elements derived from pMON6458 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, AraBAD promoter, g10L ribosome binding site and lamB secretion leader. Plasmid pMON5978 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON5976 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI,HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and screened for the restriction sites EcoRV and NheI and DNA sequenced to confirm the correct insert.

EXAMPLE 9

Construction of pMON5988

The plasmid DNA of pMON5987 was digested with NheI and EcoRI, resulting in a 3903 base pair NheI, EcoRI fragment. The 3903 base pair NheI, EcoRI fragment was ligated to 1.0 picomoles of the following annealed oligonucleotides (Oligo #3 and Oligo #4):

5'-CTAGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA-
3'-GGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTATAGTT-

GGACGGTGACTGGAATG-3' [SEQ ID NO:131]
CCTGCCACTGACCTTACAATT-5' [SEQ ID NO:132]

The ligation reaction mixture was used to transform

E. coli K-12 strain JM101 and transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and sequenced to confirm positive clones. This plasmid was constructed to change alanine 101 to aspartic acid in the hIL-3 gene (15-125). The Ala^{101} to Asp^{101} change was confirmed by DNA sequencing. This plasmid was designated pMON5988 and encodes Peptide #1 [SEQ ID NO:65].

EXAMPLE 10

Construction of pMON5873 which encodes [Met-(1-133)hIL-3]

The gene obtained from British Biotechnology, Ltd. specified arginine at codon position 129. The amino acid specified in the native hIL-3 cDNA is serine. To produce a protein with the native sequence at this position, the portion of the coding sequence between the EcoRI site at codons 106 and 107 and the NheI site at codons 129 and 130 was replaced. Plasmid DNA of pMON5854 (Example 3) and pMON5853 (Example 64) were treated with EcoRI and NheI. The larger fragments of each were gel purified. These were ligated to a pair of an annealed oligonucleotides with the following sequences:

5'-AATTCCGTCGTAAACTGACCTTCTATCTGAAAACC-
3'-GGCAGCATTTGACTGGAAGATAGACTTTTGG-

TTGGAGAACGCGCAGGCTCAACAGACCACTCTGTCG-3' [SEQ ID NO: 136]
AACCTCTTGCGCGTCCGAGTTGTCTGGTGAGACAGCGATC-5' [SEQ ID NO:137]

The ligation reaction mixtures were used to transform competent JM101 cells to ampicillin resistance. Colonies were picked into broth and grown. Plasmid DNA was isolated and screened for the presence of a new StyI recognition site present in the synthetic DNA and not in pMON5854 and pMON5853. The nucleotide sequence of the gene in the region between EcoRI and NheI was determined

and found to be that of the synthetic oligonucleotides. The new plasmids were designated pMON5873 encoding [Met-(1-133)hIL-3] and pMON5872 encoding [Met-(15-133)hIL-3].

The plasmid, pMON5873, encodes Met-(1-133)hIL-3 which has the following amino acid sequence:
Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser
Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser
Leu Ala Ile Phe [SEQ ID NO:128]

EXAMPLE 11

Construction of pMON6458

Plasmid pMON6525 DNA was digested with restriction enzymes HindIII and SalI and the resulting 3172 base pair fragment was isolated from a 1% agarose gel by interception onto DEAE membrane. The genetic elements derived from pMON6525 are the beta-lactamase gene (AMP), pBR327 origin of replication, and phage f1 origin of replication as the transcription terminator. (The genetic elements derived from plasmid pMON6525 are identical to those in plasmid pMON2341 which could also be used to construct pMON6458.) Plasmid pMON6457 was digested with restriction enzymes HindIII and SalI and the resulting 1117 base pair fragment was isolated by PAGE and crush and soak elution. The genetic elements derived from pMON6457 are the pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and the (15-125) hIL-3 gene. The restriction fragments were ligated

and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Clones containing the hIL-3 gene (encoding amino acids 15-125) contained a 345 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6458. This plasmid was constructed to eliminate an EcoRI restriction site outside the hIL-3 gene coding region in plasmid pMON6457.

EXAMPLE 12

Construction of pMON6455

Plasmid pMON5905 DNA was digested with restriction enzymes HindIII and NcoI resulting in a 3936 base pair fragment. The genetic elements derived from pMON5905 are the beta-lactamase gene (AMP), pBR327 origin of replication, pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and phage f1 origin of replication as the transcription terminator. The following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, g10L ribosome binding site and phage f1 origin of replication as the transcription terminator, derived from plasmid pMON5905 are identical to those in plasmid pMON5594 which could also be used to construct pMON6455. The AraBAD promoter is identical to that described in pMON6235. The lamB signal peptide sequence used in pMON6455 is that shown in Figure 8 fused to hIL-3 (15-125) at the NcoI site. Plasmid pMON5887 DNA was digested with restriction enzymes HindIII and NcoI, resulting in a 384 base pair NcoI, HindIII fragment. The restriction fragments were ligated, and the ligation reaction mixture was used to transform into E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated

and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Positive clones containing the hIL-3 gene (encoding amino acids 1-125) contained a 384 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6455.

EXAMPLE 13

Construction of pMON6456

Plasmid pMON5905 DNA was digested with restriction enzymes HindIII and NcoI resulting in a 3936 base pair fragment. The genetic elements derived from pMON5905 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site and the lamB secretion leader. Plasmid pMON5871 was digested with restriction enzymes HindIII and NcoI, resulting in a 330 base pair NcoI, HindIII fragment. The genetic element derived from pMON5871 encompassed the bases encoding the (1-107) hIL-3 gene. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Clones containing the hIL-3 gene (encoding amino acids 1-107) contained a 330 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6456.

EXAMPLE 14

Construction of pMON6457

Plasmid pMON6455 DNA grown in E. coli strain GM48

(dam-)was digested with restriction enzymes NcoI and ClaI, resulting in a 4263 base pair NcoI, ClaI fragment. The restriction fragment was ligated to 1.0 picomoles of annealed oligonucleotides (Oligo #5 and Oligo #6) with the following sequence coding for Met Ala 14-20 hIL-3:

5'-CATGGCTAACTGCTCTAACATGAT-3'[SEQ ID NO:151]
3'-CGATTGACGAGATTGTACTAGC-5'[SEQ ID NO:152]

The resulting DNA was transformed into E. coli K-12 strain JM101 and transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes XbaI and EcoRI in double digest. Positive clones containing the hIL-3 gene (encoding aa 15-125 of hIL-3) contained a 433 base pair XbaI, EcoRI restriction fragment and were DNA sequenced. This construct was designated pMON6457. This plasmid was constructed to delete the first 14 amino acids of hIL-3. The coding sequence of the resulting gene begins as follows:

5' ATG GCT AAC TGC... 3' [SEQ ID NO:153]
Met Ala Asn Cys... [SEQ ID NO:154]
15

The first two amino acids (Methionine, Alanine) create an NcoI restriction site and a signal peptidase cleavage site between the lamB signal peptide and (15-125) hIL-3. Plasmid pMON6457 encodes (15-125) hIL-3 which has the amino acid sequence designated SEQ ID NO:65.

EXAMPLE 15

Construction of pMON6235

One of the DNA fragments used to create this plasmid was generated by site-directed mutagenesis employing PCR

techniques described previously using the following oligonucleotides, Oligo #51(A) [SEQ ID NO:155] and Oligo #52(A) [SEQ ID NO:156], were used as primers in this procedure. The template for the PCR reaction was E. coli strain W3110 chromosomal DNA, prepared as described in Maniatis (1982). The oligonucleotide primers were designed to amplify the AraBAD promoter (Greenfield et al., 1978). The resulting DNA product was digested with the restriction enzymes SacII and BglII. The reaction mixture was purified as described previously. Plasmid, pMON5594, DNA was digested with SacII and BglII, resulting in a 4416 base pair SacII,BglII restriction fragment which contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, G10L ribosome binding site, phage f1 origin of replication as the transcription terminator and the chloramphenicol acetyl transferase (cat) gene. The 4416 base pair SacII,BglII restriction fragment from pMON5594 was ligated to the PCR-generated SacII, BglII DNA fragment. The ligation mixture was used to transform E. coli K-12 strain JM101. Positive clones contained a 323 base pair SacII,BglII fragment and were DNA sequenced to confirm that the SacII,BglII fragment was the AraBAD promoter. This construct was designated pMON6235.

EXAMPLE 16

Construction of pMON6460

One of the DNA fragments to construct this plasmid was generated by site-directed mutagenesis employing PCR techniques described previously using the oligonucleotides, Oligo #7 [SEQ ID NO: 26] and Oligo #8 [SEQ ID NO: 27] as primers. The template for the PCR reaction was plasmid pMON6458 DNA. The resulting DNA product was digested with the restriction enzymes *NcoI* and *EcoRI*. Upon completion, the digest was heated at 70°C for 15 minutes to inactivate the enzymes. The

restriction fragment was purified by phenol/chloroform extraction and precipitation with equal volume isopropanol in the presence of 2M NH_4OAc. The oligonucleotide, Oligo #8, introduces two stop codons (TAA) after amino acid 93 of hIL-3 and creates a *SalI* restriction endonuclease recognition sequence. The *NcoI, EcoRI* restriction fragment from pMON6458 was ligated to the PCR-generated *NcoI, EcoRI* restriction fragment. Positive clones containing the above mentioned changes released a 1023 base pair *SalI* fragment. This construct was designated pMON6460. This plasmid was constructed to serve as the template for the creation of single amino acid substitution variants at positions 94, 95, 96 and 97 of hIL-3.

EXAMPLE 17

Construction of pMON6461

One of the DNA fragments to create this plasmid was generated by site-directed mutagenesis employing PCR techniques described previously using the following oligonucleotide, Oligo #7 [SEQ. ID NO: 26] and Oligo #9 [SEQ. ID NO: 28], as primers. The template for the PCR reaction was plasmid pMON6458 DNA. The resulting DNA product was digested with the restriction enzymes *NcoI* and *EcoRI*. The oligonucleotide, Oligo #9, introduces two stop codons (TAA) after amino acid 97 of hIL-3 and creates a *SalI* restriction endonuclease recognition sequence. The *NcoI, EcoRI* restriction fragment from pMON5458 was ligated to the PCR-generated *NcoI, EcoRI* DNA fragment. Positive clones containing the above mentioned changes released a 1035 base pair *SalI* fragment. This construct was designated pMON6461. This plasmid was constructed to serve as the template for the creation of single amino acid substitution variants at positions 98, 99, 100 and 101 of hIL-3.

EXAMPLE 18

Construction of pMON6462

One of the DNA fragments to create this plasmid was generated by site-directed mutagenesis employing PCR techniques described previously using the following oligonucleotide, Oligo #7 [SEQ. ID NO: 26] and Oligo #10 [SEQ. ID NO: 31], as primers. The template for the PCR reaction was plasmid pMON6458 DNA. The resulting DNA product was digested with the restriction enzymes *NcoI* and *EcoRI*. The oligonucleotide, Oligo #10 [SEQ. ID NO: 31] introduces two stop codons (TAA) after amino acid 101 of hIL-3 and creates a *SalI* restriction endonuclease recognition sequence. The *NcoI, EcoRI* restriction fragment from pMON5458 was ligated to the PCR-generated *NcoI, EcoRI* DNA fragment. Positive clones containing the above mentioned changes released a 1047 base pair *SalI* fragment. This construct was designated pMON6462. This plasmid was constructed to serve as the template for the creation of single amino acid substitution variants at positions 102, 103, 104 and 105 of hIL-3.

EXAMPLE 19

Construction of single amino acid substitution libraries at positions 94, 95, 96 and 97

One of the DNA fragments used to construct the plasmids containing single amino acid substitution at positions 94, 95, 96 and 97 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction plasmid pMON6460 DNA was the template and the oligonucleotide, Oligo #7 [SEQ. ID NO: 26], was used as the primer at the N-terminus. The degenerate oligonucleotides, Oligo #11 [SEQ. ID NO: 32], Oligo #12 [SEQ. ID NO: 33], Oligo #13 [SEQ. ID NO: 34] and Oligo #14 [SEQ. ID NO: 35], were the primers at the

C-terminus. These oligonucleotides are 32-fold degenerate, with G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 94, 95, 96 and 97 of hIL-3 respectively. These degenerate oligonucleotide primers theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at a single position. The degenerate oligonucleotides (Oligo #11 [SEQ. ID NO: 32], Oligo #12 [SEQ. ID NO: 33], Oligo #13 [SEQ. ID NO: 34] and Oligo #14 [SEQ. ID NO: 35]) replace the twelve bases introduced into pMON6460, that encode the two stop codons (TAA) after amino acid 93 of hIL-3 and the *SalI* recognition sequence. At the other 9 bases the DNA sequence was restored to encode the native hIL-3 protein sequence. The resulting PCR-generated DNA products were digested with the restriction enzymes *NcoI* and *EcoRI*. The 4008 bp *NcoI*, *EcoRI* restriction fragment from pMON6460 was ligated to the PCR-generated *NcoI*, *EcoRI* DNA fragments. Plasmid DNA from individual colonies was isolated as described previously and screened by DNA dot blot differential hybridization using the oligonucleotide, Oligo #15 [SEQ. ID NO: 36], as the probe which had been labeled with P^{32}. Clones shown to be positive by hybridization were selected, plasmid DNA isolated and DNA sequenced to determine the amino acid substitution.

EXAMPLE 20

Construction of single amino acid substitution libraries at positions 98, 99, 100 and 101

Single amino acid substitutions variants were constructed at position 98, 99, 100 and 101 as described previously, with the following changes. In the PCR reaction the template was plasmid pMON6461 DNA and the oligonucleotide, Oligo #7 [SEQ. ID NO: 26], was used as the primer at the N-terminus. The degenerate

oligonucleotides, Oligo #16 [SEQ. ID NO: 37], Oligo #17 [SEQ. ID NO: 38], Oligo #18 [SEQ. ID NO: 39] and Oligo #19 [SEQ. ID NO: 40], were used as primers at the C-terminus. The resulting PCR-generated DNA products were purified and digested with restriction enzymes *NcoI* and *EcoRI*. The 4008 bp *NcoI*, *EcoRI* restriction fragment from pMON6461 was ligated to the PCR-generated DNA *NcoI*, *EcoRI* restriction fragment. Single colonies were screened by DNA dot blot differential hybridization using the oligonucleotide, Oligo #20 [SEQ. ID NO: 41], as the probe. Clones shown to be positive by hybridization were selected, plasmid DNA isolated and DNA sequenced to determine the amino acid substitution.

EXAMPLE 21

Construction of single amino acid substitution libraries at positions 102, 103, 104 and 105

Single amino acid substitutions variants were constructed at position 102, 103, 104 and 105 as described previously, with the following changes. The template was pMON6462 and the oligonucleotide, Oligo #7 [SEQ. ID NO: 26], was used as the primer at the N-terminus. The degenerate oligonucleotides, Oligo #21 [SEQ. ID NO: 42], Oligo #22 [SEQ. ID NO: 43], Oligo #23 [SEQ. ID NO: 44] and Oligo #24 [SEQ. ID NO: 45] were used as primers at the C-terminus. The resulting PCR-generated DNA products were purified and digested with restriction enzymes, *NcoI* and *EcoRI*. The 4008 bp *NcoI*, *EcoRI* restriction fragment from pMON6462 was ligated to the PCR-generated *NcoI*, *EcoRI* restriction fragment. Single colonies were screened by DNA dot blot differential hybridization using the oligonucleotide, Oligo #25 [SEQ. ID NO: 46], as the probe. Clones shown to be positive by hybridization were selected, plasmid DNA isolated and DNA sequenced to determine the amino acid substitution.

EXAMPLE 22

Construction of plasmid pMON6464

Amino acids 17-22 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON6458 DNA was the template in the PCR reaction using the oligonucleotides, Oligo #26 and Oligo #27 as primers. The resulting PCR-generated DNA products were purified and digested with *NcoI* and *EcoRI*. The 4008 bp *NcoI, EcoRI* restriction fragment from pMON6458 was ligated to the PCR-generated *NcoI, EcoRI* restriction fragment. Positive clones contained a 263 base pair *NcoI, EcoRI* restriction fragment in which the bases encoding amino acids 17-22 of hIL-3 have been deleted. pMON6464 was made to serve as the template for the creation of single amino acid substitution variants at positions 17, 18, 19, 20, 21 and 22 of hIL-3.

EXAMPLE 23

Construction of plasmid pMON6465

Amino acids 23-28 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON6458 DNA was the template in the reaction using the oligonucleotides, Oligo # 26 and Oligo #28, as primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *EcoRI*. The 4008 bp *NcoI, EcoRI* restriction fragment from pMON6458 was ligated to the PCR-generated *NcoI, EcoRI* restriction fragment. Positive clones contained a 263 base pair *NcoI, EcoRI* restriction fragment in which the bases encoding amino acids 23-28 of hIL-3 have been deleted. pMON6465 was made to serve as the template for the creation of single amino acid substitution variants at positions 23, 24, 25, 26, 27 and 28 of hIL-3.

EXAMPLE 24

Construction of plasmid pMON6466

Amino acids 29-34 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON6458 DNA was the template in the reaction using the oligonucleotides, Oligo #26 and Oligo #29 as the primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *EcoRI*. The 4008 bp *NcoI, EcoRI* restriction fragment from pMON6458 was ligated to the PCR-generated *NcoI, EcoRI* restriction fragment. Positive clones contained a 263 base pair *NcoI, EcoRI* restriction fragment in which the bases encoding amino acids 29-34 of hIL-3 have been deleted. pMON6466 was made to serve as the template for the creation of single amino acid substitution variants at positions 29, 30, 31, 32, 33 and 34 of hIL-3.

EXAMPLE 25

Construction of plasmid pMON6467

Amino acids 35-40 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON5988 DNA was the template in the reaction using the oligonucleotides, Oligo #7 and Oligo #30, as primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *EcoRV*. The *NcoI, EcoRV* restriction fragment from pMON5988 was ligated to the PCR-generated *NcoI, EcoRV* restriction fragment. Positive clones contained a 81 base pair *NcoI, EcoRV* restriction fragment in which the bases encoding amino acids 35-40 of hIL-3 have been deleted. pMON6467 was made to serve as the template for the creation of single amino acid substitution variants at positions 35, 36, 37, 38, 39 and 40 of hIL-3.

EXAMPLE 26

Construction of plasmid pMON6468

Amino acids 41-46 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON5988 DNA was the template in the reaction using the oligonucleotides, Oligo #7 and Oligo #31, as the primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *XhoI*. The *NcoI*, *XhoI* restriction fragment from pMON5988 was ligated to the PCR-generated *NcoI*, *XhoI* restriction fragment. Positive clones contained a 119 base pair *NcoI*, *XhoI* restriction fragment in which the bases encoding amino acids 41-46 of hIL-3 have been deleted. pMON6468 was made to serve as the template for the creation of single amino acid substitution variants at positions 41, 42, 43, 44, 45 and 46 of hIL-3.

EXAMPLE 27

Construction of plasmid pMON6469

Amino acids 47-52 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON5988 DNA was the template in the reaction using the oligonucleotides, Oligo #7 and Oligo #32, as the primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *XhoI*. The *NcoI*, *XhoI* restriction fragment from pMON5988 was ligated to the PCR-generated *NcoI*, *XhoI* restriction fragment. Positive clones contained a 119 base pair *NcoI*, *XhoI* restriction fragment in which the bases encoding amino acids 47-52 of hIL-3 have been deleted. pMON6469 was made to serve as the template for the creation of single amino acid substitution variants at positions 47, 48, 49, 50, 51 and 52 of hIL-3.

EXAMPLE 28

Construction of plasmid pMON6470

Amino acids 53-58 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid, pMON5988, DNA was the template in the reaction using the oligonucleotides, Oligo # 7 and Oligo #33, as primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *NsiI*. The *NcoI*, *NsiI* restriction fragment from pMON5988 was ligated to the PCR-generated *NcoI*, *NsiI* restriction fragment. Positive clones contained a 152 base pair *NcoI*, *NsiI* restriction fragment in which the bases encoding amino acids 53-58 of hIL-3 have been deleted. pMON6470 was made to serve as the template for the creation of single amino acid substitution variants at positions 53, 54, 55, 56, 57 and 58 of hIL-3.

EXAMPLE 29

Construction of plasmid pMON6471

Amino acids 59-64 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON5988 DNA was the template in the reaction using the oligonucleotides, Oligo #7 and Oligo #34, as the primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *NsiI*. The *NcoI*, *NsiI* restriction fragment from pMON5988 was ligated to the PCR-generated *NcoI*, *NsiI* restriction fragment. Positive clones contained a 152 base pair *NcoI*, *NsiI* restriction fragment in which the bases encoding amino acids 59-64 of hIL-3 have been deleted. pMON6471 was made to serve as the template for the creation of single amino acid substitution variants at positions 59, 60, 61, 62, 63 and 64 of hIL-3.

EXAMPLE 30

Construction of plasmid pMON6472

Amino acids 65-70 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON5988 DNA was the template in the reaction using the oligonucleotides, Oligo # 26 and Oligo # 35, as primers. The resulting PCR-generated DNA product was purified and digested with *EcoRI* and *XhoI*. The *EcoRI*, *XhoI* restriction fragment from pMON5988 was ligated to the PCR-generated *EcoRI*, *XhoI* restriction fragment. Positive clones contained a 126 base pair *EcoRI*, *XhoI* restriction fragment in which the bases encoding amino acids 65-70 of hIL-3 have been deleted. pMON6472 was made to serve as the template for the creation of single amino acid substitution variants at positions 65, 66, 67, 68, 69 and 70 of hIL-3.

EXAMPLE 31

Construction of plasmid pMON6473

Amino acids 71-76 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid, pMON5988, DNA was the template in the reaction using the oligonucleotides, Oligo #26 and Oligo #36, as primers. The resulting PCR-generated DNA product was and digested with *PstI* and *EcoRI*. The *PstI*, *EcoRI* restriction fragment from pMON5988 was ligated to the PCR-generated *PstI*, *EcoRI* restriction fragment. Restriction analysis was with *NcoI*, *NsiI* and *EcoRI* in a triple digest. Positive clones contained a 263 base pair *NcoI*, *EcoRI* restriction fragment, in which the bases encoding amino acids 71-76 of hIL-3 have been deleted, and lost the *NsiI* restriction site. pMON6473 was made to serve as the template for the creation of single amino acid substitution variants at positions 71, 72, 73, 74,

75 and 76 of hIL-3.

EXAMPLE 32

Construction of plasmid pMON6474

Amino acids 77-82 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON5988 DNA was the template in the reaction using the oligonucleotides, Oligo #26 and Oligo #37, as primers. The resulting PCR-generated DNA product was purified and digested with *PstI* and *EcoRI*. The *PstI*, *EcoRI* restriction fragment from pMON5988 was ligated to the PCR-generated *PstI*, *EcoRI* restriction fragment. Restriction analysis was with *NcoI*, *NsiI* and *EcoRI* in a triple digest. Positive clones contained a 170 base pair *NcoI*, *NsiI* restriction fragment and a 93 base pair *NsiI*, *EcoRI* restriction fragment in which the bases encoding amino acids 77-82 of hIL-3 have been deleted. pMON6474 was made to serve as the template for the creation of single amino acid substitution variants at positions 77, 78, 79, 80, 81 and 82 of hIL-3.

EXAMPLE 33

Construction of plasmid pMON6475

Amino acids 83-88 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON5988 DNA was the template in the reaction using the oligonucleotides, Oligo #26 and Oligo #38, as primers. The resulting PCR-generated DNA product was digested with *PstI* and *EcoRI*. The *PstI*, *EcoRI* restriction fragment from pMON5988 was ligated to the PCR-generated *PstI*, *EcoRI* restriction fragment. Restriction analysis was with *NcoI*, *NsiI* and *EcoRI* in a triple digest. Positive clones contained a 170 base pair *NcoI*, *NsiI* restriction fragment and a 93 base pair *NsiI*,

EcoRI restriction fragment in which the bases encoding amino acids 83-88 of hIL-3 have been deleted. pMON6475 was made to serve as the template for the creation of single amino acid substitution variants at positions 83, 84, 85, 86, 87 and 88 of hIL-3.

EXAMPLE 34

Construction of plasmid pMON6476

Amino acids 88-93 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON6458 DNA was the template in the reaction using the oligonucleotides, Oligo #7 and Oligo #39, as primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *EcoRI*. The *NcoI*, *EcoRI* restriction fragment from pMON6458 was ligated to the PCR-generated *NcoI*, *EcoRI* restriction fragment. Positive clones contained a 263 base pair *NcoI*, *EcoRI* restriction fragment in which the bases encoding amino acids 88-93 of hIL-3 have been deleted. pMON6476 was made to serve as the template for the creation of single amino acid substitution variants at positions 88, 89, 90, 91, 92 and 93 of hIL-3.

EXAMPLE 35

Construction of plasmid pMON6477

Amino acids 106-111 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON6458 DNA was the template in the reaction using the oligonucleotides, Oligo #7 and Oligo #40, as primers. The resulting PCR-generated DNA fragment was purified and digested with *NcoI* and *HindIII*. The *NcoI*, *HindIII* restriction fragment from pMON6458 was ligated to the PCR-generated *NcoI*, *HindIII* restriction fragment. Positive clones contained a 327 base pair *NcoI*, *HindIII*

restriction fragment in which the bases encoding amino acids 106-111 of hIL-3 have been deleted. pMON6477 was made to serve as the template for the creation of single amino acid substitution variants at positions 106, 107, 108, 109, 110 and 111 of hIL-3.

EXAMPLE 36

Construction of plasmid pMON6478

Amino acids 112-117 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON6458 DNA was the template in the reaction using the oligonucleotides, Oligo #7 and Oligo #41, as primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *HindIII*. The 4008 bp *NcoI, HindIII* restriction fragment from pMON6458 was ligated to the PCR-generated *NcoI, HindIII* restriction fragment. Positive clones contained a 327 base pair *NcoI, HindIII* restriction fragment in which the bases encoding amino acids 112-117 of hIL-3 have been deleted. pMON6478 was made to serve as the template for the creation of single amino acid substitution variants at positions 112, 113, 114, 115, 116 and 117 of hIL-3.

EXAMPLE 37

Construction of plasmid pMON6479

Amino acids 118-123 of hIL-3 were deleted using site-directed PCR mutagenesis methods described previously. Plasmid pMON6458 DNA was the template in the reaction using the oligonucleotides, Oligo #7 and Oligo #42, as primers. The resulting PCR-generated DNA product was purified and digested with *NcoI* and *HindIII*. The *NcoI, HindIII* restriction fragment from pMON6458 was ligated to the PCR-generated *NcoI, HindIII* restriction fragment. Positive clones contained a 327 base pair *NcoI, HindIII*

restriction fragment in which the bases encoding amino acids 118-123 of hIL-3 have been deleted. pMON6479 was made to serve as the template for the creation of single amino acid substitution variants at positions 118, 119, 120, 121, 122 and 123 of hIL-3.

EXAMPLE 38

Construction of single amino acid substitution libraries at positions 17, 18, 19, 20, 21 and 22

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 17, 18, 19, 20, 21 and 22 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6464 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #43, Oligo #44, Oligo #45, Oligo #46, Oligo #47 and Oligo #48 were the primers at the C-terminus. The oligonucleotide, Oligo #26, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6464. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 17, 18, 19, 20, 21 and 22 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA product was digested with *NcoI* and *EcoRV*. Plasmid pMON6464 DNA was digested with restriction enzymes *NcoI* and *EcoRV* resulting in a 4190 base pair fragment which was ligated to the PCR-generated *NcoI*, *EcoRV* restriction fragments. Plasmid DNA was isolated and screened by DNA dot blot differential

hybridization using the oligonucleotide probe, Oligo #139, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA sequenced to determine the amino acid substitution.

EXAMPLE 39

Construction of single amino acid substitution libraries at positions 23, 24, 25, 26, 27 and 28

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 23, 24, 25, 26, 27 and 28 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6465 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #49, Oligo #50, Oligo #51, Oligo #52, Oligo #53 and Oligo #54 were the primers at the C-terminus. The oligonucleotide, Oligo #26, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6465. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 23, 24, 25, 26, 27 and 28 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with restriction enzymes *NcoI* and *EcoRV*. Plasmid pMON6465 DNA was digested with restriction enzymes *NcoI* and *EcoRV* and the resulting 4190 base pair fragment was ligated to the PCR-generated *NcoI*, *EcoRV* DNA fragments. Transformant bacteria were screened by DNA

dot blot differential hybridization using the oligonucleotide probe, Oligo #140, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA sequenced to determine the amino acid substitution.

EXAMPLE 40

Construction of single amino acid substitution libraries at positions 29, 30, 31, 32, 33 and 34

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 29, 30, 31, 32, 33 and 34 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6466 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #55, Oligo #56, Oligo #57, Oligo #58, Oligo #59 and Oligo #60 were the primers at the C-terminus. The oligonucleotide Oligo #26 was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6466. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 29, 30, 31, 32, 33 and 34 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *NcoI* and *EcoRV*. Plasmid pMON6466 DNA was digested with restriction enzymes *NcoI* and *EcoRV* and the resulting 4190 base pair fragment was ligated to the PCR-generated *NcoI*, *EcoRV* DNA fragments. Transformant bacteria were screened by DNA

dot blot differential hybridization using the oligonucleotide probe, Oligo #141, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA sequenced to determine the amino acid substitution.

EXAMPLE 41

Construction of single amino acid substitution libraries at positions 35, 36, 37, 38, 39 and 40

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 35, 36, 37, 38, 39 and 40 of hIL-3 were generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6467 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #61, Oligo #62, Oligo #63, Oligo #64, Oligo #65 and Oligo #66 were the primers at the C-terminus. The oligonucleotide, Oligo #7, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6467. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 35, 36, 37, 38, 39 and 40 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored and at the other position, 32 different codons substitutions were created at positions independently. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *NcoI* and *EcoRV*. Plasmid pMON6467 DNA was digested with restriction enzymes *NcoI* and *EcoRV* and the resulting 4190 base pair fragment was ligated to the PCR-generated *NcoI*, *EcoRV* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #142, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA sequenced to

determine the amino acid substitution.

EXAMPLE 42

Construction of single amino acid substitution libraries at positions 41, 42, 43, 44, 45 and 46

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 41, 42, 43, 44, 45 and 46 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6468 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #67, Oligo #68, Oligo #69, Oligo #70, Oligo #71 and Oligo #72 were the primers at the C-terminus. The oligonucleotide, Oligo #7, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6468. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 41, 42, 43, 44, 45 and 46 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *NcoI* and *XhoI*. Plasmid pMON6468 DNA was digested with restriction enzymes *NcoI* and *XhoI* and the resulting 4152 base pair fragment was ligated to the PCR-generated *NcoI*, *XhoI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #143, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA

sequenced to determine the amino acid substitution.

EXAMPLE 43

Construction of single amino acid substitution libraries at positions 47, 48, 49, 50, 51 and 52

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 47, 48, 49, 50, 51 and 52 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6469 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #73, Oligo #74, Oligo #75, Oligo #76, Oligo #77 and Oligo #78 , were the primers at the C-terminus. The oligonucleotide, Oligo #7, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6469. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 47, 48, 49, 50, 51 and 52 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *NcoI* and *XhoI*. Plasmid pMON6469 DNA was digested with restriction enzymes *NcoI* and *XhoI* and the resulting 4152 base pair fragment was ligated to the PCR-generated *NcoI*, *XhoI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #144, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA

sequenced to determine the amino acid substitution.

EXAMPLE 44

Construction of single amino acid substitution libraries at positions 53, 54, 55, 56, 57 and 58

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 53, 54, 55, 56, 57 and 58 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6470 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #79, Oligo #80, Oligo #81, Oligo #82, Oligo #83 and Oligo #84 , were the primers at the C-terminus. The oligonucleotide, Oligo #7, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6470. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 53, 54, 55, 56, 57 and 58 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *NcoI* and *NsiI*. Plasmid pMON6470 DNA was digested with restriction enzymes *NcoI* and *NsiI* and the resulting 4119 base pair fragment was ligated to the PCR-generated *NcoI*, *NsiI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #145, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA

sequenced to determine the amino acid substitution.

EXAMPLE 45

Construction of single amino acid substitution libraries at positions 59, 60, 61, 62, 63 and 64

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 59, 60, 61, 62, 63 and 64 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6471 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #85, Oligo #86, Oligo #87, Oligo #88, Oligo #89 and Oligo #90 , were the primers at the C-terminus. The oligonucleotide, Oligo #7, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6471. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 59, 60, 61, 62, 63 and 64 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *NcoI* and *NsiI*. Plasmid pMON6471 DNA was digested with restriction enzymes *NcoI* and *NsiI* and the resulting 4119 base pair fragment was ligated to the PCR-generated *NcoI, NsiI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #146, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA

sequenced to determine the amino acid substitution.

EXAMPLE 46

Construction of single amino acid substitution libraries at positions 65, 66, 67, 68, 69 and 70

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 65, 66, 67, 68, 69 and 70 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6472 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #91, Oligo #92, Oligo #93, Oligo #94, Oligo #95 and Oligo #96 , were the primers at the N-terminus. The oligonucleotide, Oligo #26, was used as the primer at the C-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6472. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 65, 66, 67, 68, 69 and 70 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *EcoRI* and *XhoI*. Plasmid pMON6472 DNA was digested with restriction enzymes *EcoRI* and *XhoI* and the resulting 4145 base pair fragment was ligated to the PCR-generated *EcoRI*, *XhoI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #147, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA

sequenced to determine the amino acid substitution.

EXAMPLE 47

Construction of single amino acid substitution libraries at positions 71, 72, 73, 74, 75 and 76

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 71, 72, 73, 74, 75 and 76 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6473 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #97, Oligo #98, Oligo #99, Oligo #100, Oligo #101 and Oligo #102 , were the primers at the N-terminus. The oligonucleotide, Oligo #26, was used as the primer at the C-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6473. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 71, 72, 73, 74, 75 and 76 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA fragments were purified and digested with the restriction enzymes *EcoRI* and *PstI*. Plasmid pMON6473 DNA was digested with restriction enzymes *EcoRI* and *PstI* and the resulting 4171 base pair fragment was ligated to the PCR-generated *EcoRI*, *PstI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #148, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA

sequenced to determine the amino acid substitution.

EXAMPLE 48

Construction of single amino acid substitution libraries at positions 77, 78, 79, 80, 81 and 82

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 77, 78, 79, 80, 81 and 82 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the reaction the plasmid pMON6474 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #103, Oligo #104, Oligo #105, Oligo #106, Oligo #107 and Oligo #108 , were the primers at the N-terminus. The oligonucleotide, Oligo #26, was used as the primer at the C-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6474. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 77, 78, 79, 80, 81 and 82 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *EcoRI* and *PstI* as described previosly. Plasmid pMON6474 DNA was digested with restriction enzymes *EcoRI* and *PstI* and the resulting 4171 base pair fragment was ligated to the PCR-generated *EcoRI, PstI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #149, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA

sequenced to determine the amino acid substitution.

EXAMPLE 49

Construction of single amino acid substitution libraries at positions 83, 84, 85, 86, 87 and 88

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 83, 84, 85, 86, 87 and 88 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6475 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #109, Oligo #110, Oligo #111, Oligo #112, Oligo #113 and Oligo #114 , were the primers at the N-terminus. The oligonucleotide, Oligo #26, was used as the primer at the C-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6475. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 83, 84, 85, 86, 87 and 88 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA producs were purified and digested with the restriction enzymes *EcoRI* and *PstI*. Plasmid pMON6475 DNA was digested with restriction enzymes *EcoRI* and *PstI* and the resulting 4171 base pair fragment was ligated to the PCR-generated *EcoRI*, *PstI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #150, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA

sequenced to determine the amino acid substitution.

EXAMPLE 50

Construction of single amino acid substitution libraries at positions 88, 89, 90, 91, 92 and 93

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 88, 89, 90, 91, 92 and 93 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6476 DNA was the template and the following degenerate oligonucleotides, Oligo #114, Oligo #115, Oligo #116, Oligo #117, Oligo #118 and Oligo #119 , were the primers at the C-terminus. The oligonucleotide, Oligo #7, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6476. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 88, 89, 90, 91, 92 and 93 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *EcoRI* and *NcoI*. Plasmid pMON6476 DNA was digested with restriction enzymes *EcoRI* and *NcoI* and the resulting 4008 base pair fragment was ligated to the PCR-generated *EcoRI, NcoI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #151, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA

sequenced to determine the amino acid substitution.

EXAMPLE 51

Construction of single amino acid substitution libraries at positions 106, 107, 108, 109, 110 and 111

One of the DNA fragments used to construct the plasmids containing the single amino acid substitutions at positions 106, 107, 108, 109, 110 and 111 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously in two sequential PCR reactions. In the first PCR reaction, plasmid pMON6477 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #120, Oligo #121, Oligo #122, Oligo #123, Oligo #124 and Oligo #125 were the primers at the C-terminus. The oligonucleotide, Oligo #7 was the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6477. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 106, 107, 108, 109, 110 and 111 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid positions the native hIL-3 DNA sequence was restored. The DNA generated in this PCR reaction was purified by phenol/chloroform extraction and precipitation with equal volume isopropanol in the presence of 2M NH_4OAc to remove any primer that was not extended. This DNA was then used as a primer in the second PCR reaction.

In the second PCR reaction plasmid pMON6477 DNA was the template, the DNA product generated in the first PCR reaction (described above) was the primer at the N-terminus and the oligonucleotide, Oligo #126 (DNA

sequence shown in Table 1), was the primer at the C-terminus. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *HindIII* and *NcoI*. Plasmid pMON6477 was digested with restriction enzymes *HindIII* and *NcoI* and the resulting 3944 base pair fragment was ligated to the PCR-generated *HindIII*, *NcoI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #152, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA sequenced to determine the amino acid substitution.

EXAMPLE 52

Construction of single amino acid substitution libraries at positions 112, 113, 114, 115, 116 and 117

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 112, 113, 114, 115, 116 and 117 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6478 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #127, Oligo #128, Oligo #129, Oligo #130, Oligo #131 and Oligo #132 , were the primers at the C-terminus. The oligonucleotide, Oligo #7, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6478. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 112, 113, 114, 115, 116 and 117 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid

positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *HindIII* and *NcoI*. Plasmid pMON6478 was digested with restriction enzymes *HindIII* and *NcoI* and the resulting 3944 base pair fragment was ligated to the PCR-generated *HindIII*, *NcoI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #153, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA sequenced to determine the amino acid substitution.

EXAMPLE 53

Construction of single amino acid substitution libraries at positions 118, 119, 120, 121, 122 and 123

One of the DNA fragments used to construct the plasmids containing single amino acid substitutions at positions 118, 119, 120, 121, 122 and 123 of hIL-3 was generated by site-directed mutagenesis employing PCR techniques described previously. In the PCR reaction the plasmid pMON6479 DNA was the template and the following 32 fold degenerate oligonucleotides, Oligo #133, Oligo #134, Oligo #135, Oligo #136, Oligo #137 and Oligo #138 , were the primers at the C-terminus. The oligonucleotide, Oligo #7, was used as the primer at the N-terminus. The degenerate oligonucleotides replace the eighteen bases, encoding six amino acids, deleted in pMON6479. The degenerate oligonucleotides have G, A, T or C in the first and second positions and G or C in the third position of a single codon at amino acid positions 118, 119, 120, 121, 122 and 123 of hIL-3 respectively. These degenerate oligonucleotide primers result in libraries which theoretically contain 32 different codons encoding all 20 amino acid substitutions and one translational stop codon at one position. At the other five amino acid

positions the native hIL-3 DNA sequence was restored. The resulting PCR-generated DNA products were purified and digested with the restriction enzymes *HindIII* and *NcoI*. Plasmid pMON6479 DNA was digested with restriction enzymes *HindIII* and *NcoI* and the resulting 3944 base pair fragment was ligated to the PCR-generated *HindIII, NcoI* DNA fragments. Transformant bacteria were screened by DNA dot blot differential hybridization using the oligonucleotide probe, Oligo #154, which had been labeled with P^{32}. Clones shown to be positive by colony hybridization were selected, plasmid DNA isolated and DNA sequenced to determine the amino acid substitution.

EXAMPLE 54

Construction of pMON13358

Plasmid pMON5978 DNA (Example 6) was digested with restriction enzymes NsiI and EcoRI and the resulting 3853 base pair NsiI,EcoRI fragment contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, recA promoter, g10L ribosome binding site and the bases encoding amino acids 15-71 and 106-125 of (15-125) hIL-3. The 3853 base pair NsiI,EcoRI restriction fragment from pMON5978 was ligated to the following annealed complementary oligonucleotides.

Oligo #15(A) [SEQ ID NO: 29]

Oligo #16(A) [SEQ ID NO: 30]

In the resulting plasmid the 111 bases between the NsiI and EcoRI restriction sites in the (15-125) hIL-3 gene are replaced with 24 bases from the above mentioned oligonucleotides. This linker also creates a NdeI recognition sequence.

EXAMPLE 55

Construction of pMON13304

Plasmid pMON13358 DNA is digested with restriction enzymes PstI and EcoRI and the resulting 3846 base pair PstI,EcoRI fragment contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, recA promoter, g10L ribosome binding site and the bases encoding amino acids 15-69 and 106-125 of (15-125) hIL-3. The 3846 base pair NsiI,EcoRI restriction fragment from pMON13358 is ligated to the following annealed complementary oligonucleotides.

Oligo #155 [SEQ ID NO:200]
Oligo #156 [SEQ ID NO:201]

Oligo #157 [SEQ ID NO:202]
Oligo #158 [SEQ ID NO:203]

Oligo #159 [SEQ ID NO:204]
Oligo #160 [SEQ ID NO:205]

Oligo #161 [SEQ ID NO:206]
Oligo #162 [SEQ ID NO:207]

When assembled, the oligonucleotides create PstI and EcoRI restriction ends and the DNA sequence that encodes amino acids 70-105 of (15-125) hIL-3 with the following amino acid substitutions; 98I and 100R. The codons encoding amino acids 70-105 of (15-125) hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13304, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

Peptide #A1 [SEQ ID NO:66]

EXAMPLE 56

Construction of pMON13305

Plasmid pMON13358 DNA is digested with restriction enzymes PstI and EcoRI and the resulting 3846 base pair PstI,EcoRI fragment contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, recA promoter, g10L ribosome binding site and the bases encoding amino acids 15-69 and 106-125 of (15-125) hIL-3. The 3846 base pair NsiI,EcoRI restriction fragment from pMON13358 is ligated to the following annealed complementary oligonucleotides.

Oligo #155 [SEQ ID NO:200]
Oligo #156 [SEQ ID NO:201]

Oligo #157 [SEQ ID NO:202]
Oligo #158 [SEQ ID NO:203]

Oligo #159 [SEQ ID NO:204]
Oligo #160 [SEQ ID NO:205]

Oligo #163 [SEQ ID NO:208]
Oligo #164 [SEQ ID NO:209]

When assembled, the oligonucleotides create PstI and EcoRI restriction ends and the DNA sequence that encodes amino acids 70-105 of (15-125) hIL-3 with the following amino acid substitutions; 95R, 98I and 100R. The codons encoding amino acids 70-105 of (15-125) hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13305, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

Peptide #A2 [SEQ ID NO:67]

EXAMPLE 57

Construction of pMON13286

Plasmid pMON5978 DNA was digested with restriction enzymes NcoI and EcoRV and the resulting 3865 base pair NcoI,EcoRV fragment contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter, g10L ribosome binding site and the bases encoding amino acids 47-125 of (15-125) hIL-3. The 3865 base pair NcoI,EcoRV restriction fragment from pMON5978 was ligated to the following annealed complementary oligonucleotides.

Oligo #165 [SEQ ID NO:210]
Oligo #166 [SEQ ID NO:211]

Oligo #167 [SEQ ID NO: 212]
Oligo #168 [SEQ ID NO:213]

Oligo #169 [SEQ ID NO: 214]
Oligo #170 [SEQ ID NO:215]

When assembled, the oligonucleotides create NcoI and EcoRV restriction ends and the DNA sequence that encodes amino acids 15-46 of (15-125) hIL-3 with the following amino acid substitutions; 42D, 45M and 46S. The codons encoding amino acids 15-46 of (15-125) hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13286, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

Peptide #A3 [SEQ ID NO:68]

DNA sequence #A4 pMON13286 42D, 45M, 46S

ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA
GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGAC GAAGACATGT
CTATCCTGAT GGAAAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC
CGTGCTGTCA AGTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA
AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC
CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC
TTCTATCTGA AAACCTTGGA GAACGCGCAG GCTCAACAG

[SEQ ID NO: 69]

EXAMPLE 58

Construction of pMON5853 (Fig 6) which encodes [Met-(15-133)hIL-3(Arg^{129})]

Plasmid DNA of pMON5847 (Example 2) was treated with NcoI. The restriction enzyme was inactivated by heat treatment (65°C for 10 minutes). The DNA was then treated with large fragment of DNA polymerase I (Klenow) in the presence of all four nucleotide precursors. This produces DNA termini with non-overlapping ends. After 5 minutes at 37°C, the polymerase was inactivated by heat treatment at 65°C for 10 minutes. The DNA was then treated with HpaI, an enzyme which produces non-overlapping termini. The DNA was ethanol precipitated and ligated. The ligation reaction mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked and plasmid DNA was analyzed by restriction analysis. A plasmid designated pMON5853 was identified as one containing a deletion of the amino terminal 14 codons of the hIL-3 gene. The DNA sequence for the junction of the ribosome binding site to the (15-133) hIL-3 gene was determined to be the following:

5'-AAGGAGATATATCCATGAACTGCTCTAAC-3' [SEQ ID NO:133]
M N C S N [SEQ ID NO:134]

The lower line contains the one-letter code for the amino acids specified by the coding sequence of the amino terminus of the 15-133 hIL-3 gene. These are methionine, asparagine, cysteine, serine and asparagine.

When cultures of JM101 cells harboring this plasmid were induced with nalidixic acid, it was found that hIL-3 (15-133) accumulated at levels higher than hIL-3 (pMON5847).

The plasmid, pMON5853, encodes Met-(15-133) hIL-3 (Arg^{129}) which has the following amino acid sequence:

Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Arg
Leu Ala Ile Phe [SEQ ID NO:135]

Formula XI shown below is a representation of a [(15-125)hIL-3 mutein] with numbers in bold type added above the amino acids to represent the position at which the amino acid below the bolded number appears in native (1-133)hIL-3 [e. g. the amino acid at position 1 of Formula XI corresponds to the Asn which appears at position 15 in native (1-133)hIL-3]. The number shown in bold indicates the amino acids that correspond to the native IL-3(1-133). The non-bold members below the amino acids sequences are for Seq Id reference numbers. When the muteins are expressed the initial amino acid may be

preceded by Met- or Met-Ala-.

```
 15                  20                  25
Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln
 1                   5                  10                  15

 30                  35                  40
Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp
                    20                  25                  30
 45                  50                  55
Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu
                    35                  40                  45

 60                  65                  70
Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile
                    50                  55                  60

 75                  80                  85
Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr
                    65                  70                  75

 90                  95                 100
Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp
                    80                  85                  90

105                 110                 115
Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu
                    95                 100                 105
120                 125
Asn Ala Gln Ala Gln Gln [SEQ ID NO:23]
                   110
```

Table 6 shows (15-125)hIL-3 muteins of the present invention which have one (and in some cases two) amino acid substitutions in the (15-125)hIL-3 polypeptide and which were constructed as described in the Examples. The mutants in Table 6 were secreted into the periplasmic space in E.coli. The periplasmic content was released by osmotic shock and the material in the crude osmotic shock fraction was screened for growth promoting activity. Biological activity is the growth promoting activity of AML cells relative to (15-125) hIL-3 (pMON6458 or pMMON5988). The numbers in parentheses indicate the number of repeat assays. When a variant was assayed more than once the standard deviation is indicated. An "-" indicates that the hIL3 variant protein level was less than 1.0 µg/ml and was not screened for growth promoting activity.

TABLE 6
(15-125) HUMAN INTERLEUKIN-3 MUTANTS

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
17/3[1]	SER	TCT	LYS	19	AAG	<0.018 (1)
17/3	SER	TCT	GLY	19	GGG	1.2 ± 1.1 (3)
17/3	SER	TCT	ASP	19	GAC	1.0 ± 0.7 (3)
17/3	SER	TCT	MET	19	ATG	0.50 (1)
17/3	SER	TCT	GLN	19	CAG	1.2 ± 0.7 (3)
17/3	SER	TCT	ARG	19	AGG	<0.070 (1)
18/4	ASN	AAC	HIS	19	CAC	1.2 ± 0.3 (3)
18/4	ASN	AAC	LEU	19	CTC	0.45 ± 0.42 (4)
18/4	ASN	AAC	ILE	19	ATC	1.5 ± 0.2 (2)
18/4	ASN	AAC	PHE	19	TTC	0.19 ± 0.26 (2)
18/4	ASN	AAC	ARG	19	CGG	0.10 (1)
18/4	ASN	AAC	GLN	19	CAA	0.37 (1)
19/5	MET	ATG	PHE	19	TTC	0.25 (1)
19/5	MET	ATG	ILE	19	ATC	0.77 ± 0.70 (9)
19/5	MET	ATG	ARG	19	AGG	0.17 (1)
19/5	MET	ATG	GLY	19	GGA	0.06 (1)
19/5	MET	ATG	ALA	19	GCG	0.19 (1)
19/5	MET	ATG	CYS	19	TGC	-
20/6	ILE	ATC	CYS	19	TGC	-
20/6	ILE	ATC	GLN	19	CAG	-
20/6	ILE	ATC	GLU	19	GAG	<0.025 (1)
20/6	ILE	ATC	ARG	19	CGC	<0.025 (1)
20/6	ILE	ATC	PRO	19	CCG	0.29 ± 0.16 (3)
20/6	ILE	ATC	ALA	19	GCG	0.18 (1)
21/7	ASP	GAT	PHE	19	TTC	<0.016 (1)
21/7	ASP	GAT	LYS	19	AAG	0.027 ±0.027 (2)
21/7	ASP	GAT	ARG	19	AGG	<0.008 (1)

[1] The first position number represents the amino acid position in (1-133)hIL-3 and the second number represents the position in (15-125)hIL-3 in which the Asn at position 15 of native hIL-3 is position 1 in (15-125)hIL-3 (See the numbering for Formula XI)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
21/7	ASP	GAT	ALA	19	GCG	0.07 ± 0.06 (3)
21/7	ASP	GAT	GLY	19	GGG	0.032 (1)
21/7	ASP	GAT	VAL	19	GTG	<0.008 (1)
22/8	GLU	GAA	TRP	19	TGG	-
22/8	GLU	GAA	PRO	19	CCG	<0.015 (1)
22/8	GLU	GAA	SER	19	TCG	<0.015 (1)
22/8	GLU	GAA	ALA	19	GCC	<0.015 (1)
22/8	GLU	GAA	HIS	19	CAC	<0.015 (1)
22/8	GLU	GAA	GLY	19	GGC	<0.008 (1)
23/9	ILE	ATT	VAL	19	GTG	0.18 (1)
23/9	ILE	ATT	ALA[2]	19	GCG	1.16 ± 0.16 (3)
23/9	ILE	ATT	LEU	19	TTG	1.3 (1)
23/9	ILE	ATT	GLY[2]	19	GGG	0.06 (1)
23/9	ILE	ATT	TRP	19	TGG	-
23/9	ILE	ATT	LYS[3]	19	AAG	-
23/9	ILE	ATT	PHE	19	TTC	-
23/9	ILE	ATT	LEU[2]	19	TTG	3.0 ± 1.1 (3)
23/9	ILE	ATT	SER[3]	19	AGC	<0.005 (1)
23/9	ILE	ATT	ARG[3]	19	CGC	-
24/10	ILE	ATA	GLY	19	GGG	<0.004 (1)
24/10	ILE	ATA	VAL	19	GTC	0.89 ± 0.23 (4)
24/10	ILE	ATA	ARG[3]	19	CGG	-
24/10	ILE	ATA	SER	19	AGC	<0.003 (1)
24/10	ILE	ATA	PHE	19	TTC	0.29 ± 0.24 (2)
24/10	ILE	ATA	LEU	19	CTG	0.52 ± 0.12 (3)
25/11	THR	ACA	HIS	19	CAC	1.11 ± 0.2 (3)
25/11	THR	ACA	GLY	19	GGC	0.48 ± 0.27 (4)
25/11	THR	ACA	GLN	19	CAG	1.0 ± 0.8 (4)
25/11	THR	ACA	ARG	19	CGG	0.26 ± 0.17 (2)
25/11	THR	ACA	PRO	19	CCG	0.36 (1)

[2] Double mutant; has PRO at position 35.

[3] Double mutant; has THR at position 49.

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
31/17	PRO	CCT	GLY	19	GGG	0.79 ± 0.61 (2)
31/17	PRO	CCT	ALA	19	GCC	0.49 (1)
31/17	PRO	CCT	ARG	19	CGC	0.25 ± 0.20 (2)
31/17	PRO	CCT	LEU	19	CTG	0.22 (1)
31/17	PRO	CCT	GLN	19	CAG	0.62 ± 0.04 (2)
31/17	PRO	CCT	LEU[4]	19	CTG	0.30 ± 0.20 (3)
32/18	LEU	TTG	VAL	19	GTG	0.01 (1)
32/18	LEU	TTG	ARG	19	CGC	1.5 ± 1.0 (4)
32/18	LEU	TTG	GLN	19	CAG	0.93 ± 0.18 (3)
32/18	LEU	TTG	ASN	19	AAC	1.2 ± 0.5 (5)
32/18	LEU	TTG	GLY[5]	19	GGC	0.84 ± 1.0 (3)
32/18	LEU	TTG	ALA	19	GCG	1.4 ± 0.7 (5)
32/18	LEU	TTG	GLU	19	GAG	0.88 ± 0.37 (2)
33/19	PRO	CC(T/C)	LEU	19	CTG	0.13 (1)
33/19	PRO	CC(T/C)	GLN	19	CAG	0.22 ± 0.20 (2)
33/19	PRO	CC(T/C)	ALA	19	GCG	0.30 ± 0.14 (2)
33/19	PRO	CC(T/C)	THR	19	ACC	<0.018 (1)
33/19	PRO	CC(T/C)	GLU	19	GAG	0.54 ± 0.43 (2)
34/20	LEU	TTG	VAL	19	GTG	1.2 ± 0.6 (3)
34/20	LEU	TTG	GLY	19	GGG	0.64 ± 0.74 (2)
34/20	LEU	TTG	SER	19	TCG	1.5 ± 0.7 (4)
34/20	LEU	TTG	LYS	19	AAG	0.97 ± 0.28 (2)
34/20	LEU	TTG	MET	19	ATG	1.7 ± 0.5 (3)
35/21	LEU	CTG	ALA	19	GCC	1.6 ± 0.5 (3)
35/21	LEU	CTG	GLY	19	GGC	<0.006 ±0.002(3)
35/21	LEU	CTG	ASN	19	AAC	1.1 ± 1.7 (5)
35/21	LEU	CTG	PRO	19	CCC	1.8 ± 2.0 (5)
35/21	LEU	CTG	GLN	19	CAA	0.98 ± 1.1 (5)
35/21	LEU	CTG	VAL	19	GTG	0.76 ± 0.86 (5)
36/22	ASP	GAC	LEU	19	CTC	0.20 (1)

[4] Double mutant; has Gly at position 32.

[5] Double mutant; has Leu at position 31.

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
25/11	THR	ACA	ALA	19	GCC	0.86 ± 0.27 (3)
26/12	HIS	CAC	THR	19	ACG	0.010 (1)
26/12	HIS	CAC	PHE	19	TTC	0.26 (1)
26/12	HIS	CAC	GLY	19	GGG	0.19 (1)
26/12	HIS	CAC	ARG	19	CGG	0.21 (1)
26/12	HIS	CAC	ALA	19	GCC	0.56 ± 0.03 (2)
26/12	HIS	CAC	TRP	19	TGG	-
27/13	LEU	TTA	GLY	19	GGG	-
27/13	LEU	TTA	ARG	19	AGG	-
27/13	LEU	TTA	THR	19	ATC	0.084 (1)
27/13	LEU	TTA	SER	19	TCC	-
27/13	LEU	TTA	ALA	19	GCG	0.01 (1)
28/14	LYS	AAG	ARG	19	CGG	0.42 ± 0.07 (2)
28/14	LYS	AAG	LEU	19	TTG	-
28/14	LYS	AAG	TRP	19	TGG	-
28/14	LYS	AAG	GLN	19	CAG	0.27 (1)
28/14	LYS	AAG	GLY	19	GGC	0.36 ± 0.07 (2)
28/14	LYS	AAG	PRO	19	CCC	0.10 ± 0.04 (2)
28/14	LYS	AAG	VAL	19	GTG	0.19 ± 0.12 (2)
29/15	GLN	CAG	ASN	19	AAC	1.62 ± 1.7 (3)
29/15	GLN	CAG	LEU	19	CTG	0.284
29/15	GLN	CAG	PRO	19	CCG	-
29/15	ARG	CAG	ARG	19	AGG	0.44 ± 0.16 (4)
29/15	GLN	CAG	VAL	19	GTG	0.62 ± 0.40 (4)
30/16	PRO	CCA	HIS	19	CAC	0.26 (1)
30/16	PRO	CCA	THR	19	ACG	0.36 (1)
30/16	PRO	CCA	GLY	19	GGG	1.2 ± 0.8 (3)
30/16	PRO	CCA	ASP	19	GAC	-
30/16	PRO	CCA	GLN	19	CAG	0.61 ± 0.37 (3)
30/16	PRO	CCA	SER	19	TCG	-
30/16	PRO	CCA	LEU	19	TTC	-
30/16	PRO	CCA	LYS	19	AAG	-
31/17	PRO	CCT	ASP	19	GAC	0.66 ± 0.71 (3)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
36/22	ASP	GAC	VAL	19	GTG	-
37/23	PHE	TTC	SER	19	AGC	0.62 ± 0.40 (4)
37/23	PHE	TTC	PRO	19	CCG	0.65 ± 0.39 (4)
37/23	PHE	TTC	TRP	19	TGG	-
37/23	PHE	TTC	ILE	19	ATC	0.1 (1)
38/24	ASN	AAC	ALA	19	GCN	1.9 (1)
40/26	LEU	CTC	TRP	19	TGG	-
40/26	LEU	CTC	ARG	19	CGC	-
41/27	ASN	AAT	CYS	19	TGC	0.18 (1)
41/27	ASN	AAT	ARG	19	CGC	0.13 ± 0.13 (2)
41/27	ASN	AAT	LEU	19	CTG	0.09 ± 0.07 (2)
41/27	ASN	AAT	HIS	19	CAC	0.49 ± 0.26 (4)
41/27	ASN	AAT	MET	19	ATG	0.30 ± 0.38 (4)
41/27	ASN	AAT	PRO	19	CCG	0.12 (1)
42/28	GLY	GGG	ASP	19	GAC	5.7 ± 5.7 (6)
42/28	GLY	GGG	SER	19	AGC	4.3 ± 4.8 (7)
42/28	GLY	GGG	CYS	19	TGC	0.53 (1)
42/28	GLY	GGG	ALA	19	GCC	5.9 ± 4.1 (7)
43/29	GLU	GAA	ASN	19	AAC	0.050 (1)
43/29	GLU	GAA	TYR	19	TAC	0.010 (1)
43/29	GLU	GAA	LEU	19	CTC	<0.009 (1)
43/29	GLU	GAA	PHE	19	TTC	<0.009 (1)
43/29	GLU	GAA	ASP	19	GAC	0.044 (1)
43/29	GLU	GAA	ALA	19	GCC	<0.009 (1)
43/29	GLU	GAA	CYS	19	TGC	<0.009 (1)
43/29	GLU	GAA	SER	19	AGC	<0.009 (1)
44/30	ASP	GAC	SER	19	TCA	0.007 (1)
44/30	ASP	GAC	LEU	19	CTG	<0.007 (1)
44/30	ASP	GAC	ARG	19	AGG	<0.007 (1)
44/30	ASP	GAC	LYS	19	AAG	<0.007 (1)
44/30	ASP	GAC	THR	19	ACG	-
44/30	ASP	GAC	MET	19	ATG	<0.007 (1)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
44/30	ASP	GAC	TRP	19	TGG	<0.007 (1)
44/30	ASP	GAC	PRO	19	CCC	<0.007 (1)
45/31	GLN	CAA	PRO	19	CCC	-
45/31	GLN	CAA	PHE	19	TTC	0.007 (1)
45/31	GLN	CAA	VAL	19	GTC	6.7 ± 6.1 (5)
45/31	GLN	CAA	MET	19	ATG	3.4 ± 1.8 (5)
45/31	GLN	CAA	LEU	19	TTG	1.1 ± 1.3 (2)
45/31	GLN	CAA	THR	19	ACG	0.96 ± 1.5 (3)
45/31	GLN	CAA	LYS	19	AAG	1.6 ± 2.2 (5)
45/31	GLN	CAA	TRP	19	TGG	0.10 (1)
46/32	ASP	GAC	PHE	19	TTC	1.2 ± 0.5 (3)
46/32	ASP	GAC	SER	19	TCC	7.9 ± 6.4 (4)
46/32	ASP	GAC	THR	19	ACC	1.8 ± 0.2 (2)
46/32	ASP	GAC	CYS	19	TGC	0.80 (1)
46/32	ASP	GAC	GLY	19	GGC	0.25 (1)
47/33	ILE	ATT	GLY	19	GGC	<0.015 (1)
47/33	ILE	ATT	VAL	19	GTG	0.38 (1)
47/33	ILE	ATT	HIS	19	CAC	0.10 (1)
47/33	ILE	ATT	SER	19	TCC	0.03 (1)
47/33	ILE	ATT	ARG	19	AGG	0.09 (1)
47/33	ILE	ATT	PRO	19	CCG	<0.015 (1)
48/34	LEU	CTG	SER	19	AGC	<0.009 (1)
48/34	LEU	CTG	CYS	19	TCG	-
48/34	LEU	CTG	ARG	19	CGC	<0.009 (1)
48/34	LEU	CTG	ILE	19	ATC	0.036 (1)
48/34	LEU	CTG	HIS	19	CAC	<0.009 (1)
48/34	LEU	CTG	PHE	19	TTC	<0.009 (1)
48/34	LEU	CTG	ASN	19	AAC	<0.009 (1)
49/35	MET	ATG	ARG	19	CGC	0.007 (1)
49/35	MET	ATG	ALA	19	GCC	0.091 (1)
49/35	MET	ATG	GLY	19	GGC	0.036 (1)
49/35	MET	ATG	PRO	19	CCC	<0.009 (1)
49/35	MET	ATG	ASN	19	AAC	0.23 (1)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
49/35	MET	ATG	HIS	19	CAC	<0.009 (1)
49/35	MET	ATG	ASP	19	GAC	0.28 ± 0.48 (3)
50/36	GLU	GAA	LEU	19	CTC	0.01 (1)
50/36	GLU	GAA	THR	19	ACC	0.20 (1)
50/36	GLU	GAA	ASP	19	GAC	-
50/36	GLU	GAA	TYR	19	TAC	0.09 (1)
50/36	GLU	GAA	GLN	19	CTG	0.02 (1)
51/37	ASN	AAT	ARG	19	CGC	2.0 ± 0.8 (3)
51/37	ASN	AAT	MET	19	ATG	0.75 ± 0.50 (2)
51/37	ASN	AAT	PRO	19	CCG	2.77 ± 1.6 (3)
51/37	ASN	AAT	SER	19	TCC	0.87 ± 0.44 (3)
51/37	ASN	AAT	THR	19	ACG	2.3 ± 1.6 (3)
51/37	ASN	AAT	HIS	19	CAC	1.3 ± 0.9 (5)
52/38	ASN	AAC	HIS	19	CAC	0.004 (1)
52/38	ASN	AAC	ARG	19	CGC	0.004 (1)
52/38	ASN	AAC	LEU	19	TGG	0.003 (1)
52/38	ASN	AAC	GLY	19	GGC	0.22 (1)
52/38	ASN	AAC	SER	19	AGC	0.07 (1)
52/38	ASN	AAC	THR	19	ACG	0.44 ± 0.30 (3)
53/39	LEU	CTT	THR	19	ACC	<0.005 (1)
53/39	LEU	CTT	ALA	19	GCG	-
53/39	LEU	CTT	GLY	19	GGC	<0.005 (1)
53/39	LEU	CTT	GLU	19	GAG	<0.005 (1)
53/39	LEU	CTT	PRO	19	CCG	<0.005 (1)
53/39	LEU	CTT	LYS	19	AAG	<0.005 (1)
53/39	LEU	CTT	SER	19	AGC	0.008 (1)
53/39	LEU	CTT	MET	19	ATG	0.31 (1)
54/40	ARG	CGA	ASP	19	GAC	<0.005 (1)
54/40	ARG	CGA	ILE	19	ATC	0.05 (1)
54/40	ARG	CGA	SER	19	TCC	0.10 (1)
54/40	ARG	CGA	VAL	19	GTG	<0.005 (1)
54/40	ARG	CGA	THR	19	ACC	0.015 (1)
54/40	ARG	CGA	GLN	19	CAG	0.04 (1)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
54/40	ARG	CGA	LEU	19	TTG	0.03 (1)
55/41	ARG	AGG	THR	19	ACC	0.65 ± 1.1 (4)
55/41	ARG	AGG	VAL	19	GTC	0.96 ± 0.36 (3)
55/41	ARG	AGG	SER	19	TCG	0.065 (1)
55/41	ARG	AGG	LEU	19	CTG	1.1 ± 1.2 (4)
55/41	ARG	AGG	GLY	19	GGC	1.0 ± 0.6 (4)
56/42	PRO	CCA	GLY	19	GGC	1.1 ± 0.8 (3)
56/42	PRO	CCA	CYS	19	TGC	0.21 (1)
56/42	PRO	CCA	SER	19	AGC	1.4 ± 0.4 (2)
56/42	PRO	CCA	GLN	19	CAG	1.8 (1)
56/42	PRO	CCA	LYS	19	AAG	0.60 (1)
57/43	ASN	AAC	GLY[6]	19	GGC	-
58/44	LEU	CTG	SER	19	AGC	<0.041 (1)
58/44	LEU	CTG	ASP	19	GAC	<0.041 (1)
58/44	LEU	CTG	ARG	19	CGG	<0.041 (1)
58/44	LEU	CTG	GLN	19	CAG	<0.041 (1)
58/44	LEU	CTG	VAL	19	GTC	<0.041 (1)
58/44	LEU	CTG	CYS	19	TGC	-
59/45	GLU	GAG	TYR	19	TAC	0.41 ± 0.37 (5)
59/45	GLU	GAG	HIS	19	CAC	0.38 ± 0.31 (2)
59/45	GLU	GAG	LEU	19	CTC	0.46 ± 0.36 (6)
59/45	GLU	GAG	PRO	19	CCC	-
59/45	GLU	GAG	ARG	19	CGC	0.15 (1)
60/46	ALA	GCA	SER	19	AGC	0.91 ± 0.55 (4)
60/46	ALA	GCA	PRO	19	CCC	-
60/46	ALA	GCA	TYR	19	TAC	<0.008 (1)
60/46	ALA	GCA	ASN	19	AAC	0.38 (1)
60/46	ALA	GCA	THR	19	ACG	0.21 (1)
61/47	PHE	TTC	ASN	19	AAC	-
61/47	PHE	TTC	GLU	19	GAG	<0.010 (1)
61/47	PHE	TTC	PRO	19	CCC	-

[6] Double mutant; has Gly at position 46.

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
61/47	PHE	TTC	LYS	19	AAG	<0.010 (1)
61/47	PHE	TTC	ARG	19	CGC	0.006 (1)
61/47	PHE	TTC	SER	19	TCG	0.17 (1)
62/48	ASN	AAC	HIS	19	CAC	-
62/48	ASN	AAC	VAL	19	GTG	0.37 ± 0.25 (4)
62/48	ASN	AAC	ARG	19	AGG	-
62/48	ASN	AAC	PRO[7]	19	CCG	1.6 ± 0.4 (3)
62/48	ASN	AAC	PRO	19	CCG	2.0 ± 0.3 (3)
62/48	ASN	AAC	THR[8]	19	ACG	2.3 ± 1.1 (3)
62/48	ASN	AAC	ASP	19	GAC	-
62/48	ASN	AAC	ILE	19	ATC	0.56 ± 0.24 (4)
63/49	ARG	A(G/A)G	TYR	19	TAC	0.47 (1)
63/49	ARG	A(G/A)G	TRP	19	TGG	0.09 (1)
63/49	ARG	A(G/A)G	LYS	19	AGG	0.52 (1)
63/49	ARG	A(G/A)G	SER[9]	19	TCC	0.13 (1)
63/49	ARG	A(G/A)G	HIS	19	CAC	0.42 ± 0.25 (7)
63/49	ARG	A(G/A)G	PRO	19	CCG	<0.014 ±0.013(2)
63/49	ARG	A(G/A)G	VAL	19	GTG	0.39 ± 0.34 (3)
64/50	ALA	GCT	ASN	19	AAC	1.5 ± 2.9 (4)
64/50	ALA	GCT	PRO	19	CCG	<0.023 (1)
64/50	ALA	GCT	SER	19	AGC	<0.023 (1)
64/50	ALA	GCT	LYS	19	AAG	<0.047 (1)
65/51	VAL	GTC	THR	19	ACC	0.71 ± 0.64 (3)
65/51	VAL	GTC	PRO	19	CCG	<0.014 (1)
65/51	VAL	GTC	HIS	19	CAC	<0.014 (1)
65/51	VAL	GTC	LEU	19	CTC	0.42 (1)
65/51	VAL	GTC	PHE	19	TTC	0.061 (1)
65/51	VAL	GTC	SER	19	TCC	0.34 (1)

[7] Double mutant; Arg at position 42.

[8] Double mutant; Phe at position 53.

[9] Double mutant; has Val at position 49.

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
66/52	LYS	AAG	ILE[10]	19	ATC	0.42 (1)
66/52	LYS	AAG	ARG	19	AGG	0.79 ± 0.18 (2)
66/52	LYS	AAG	VAL	19	GTC	0.38 ± 0.17 (2)
66/52	LYS	AAG	ASN	19	AAC	0.32 (1)
66/52	LYS	AAG	GLU	19	GAG	0.14 (1)
66/52	LYS	AAG	SER	19	TCG	0.31 (1)
66/52	LYS	AAG	VAL[11]	19	GTG	0.055 (1)
67/53	SER	AGT	ALA	19	GCG	<0.014 (1)
67/53	SER	AGT	PHE	19	TTC	1.2 ± 0.2 (2)
67/53	SER	AGT	VAL	19	GTG	0.24 (1)
67/53	SER	AGT	GLY	19	GGG	0.50 ± 0.29 (4)
67/53	SER	AGT	ASN	19	AAC	0.52 ± 0.28 (7)
67/53	SER	AGT	ILE	19	ATC	0.29 (1)
67/53	SER	AGT	PRO	19	CCG	0.055 (1)
67/53	SER	AGT	HIS	19	CAC	0.99 ± 0.62 (6)
68/54	LEU	TTA	VAL	19	GTC	0.14 (1)
68/54	LEU	TTA	TRP	19	TGG	0.07 (1)
68/54	LEU	TTA	SER	19	AGC	<0.003 (1)
68/54	LEU	TTA	ILE	19	ATC	0.84 ± 0.47 (3)
68/54	LEU	TTA	PHE	19	TTC	1.7 ± 0.3 (3)
68/54	LEU	TTA	THR	19	ACG	0.011 (1)
68/54	LEU	TTA	HIS	19	CAC	0.82 ± 0.45 (2)
69/55	GLN	CAG	ALA	19	GCG	1.2 ± 0.8 (3)
69/55	GLN	CAG	PRO	19	CCA	0.74 0.45 (4)
69/55	GLN	CAG	THR	19	ACG	0.97 ± 0.46 (4)
69/55	GLN	CAG	TRP	19	TGG	-
69/55	GLN	CAG	GLU	19	GAG	1.4 ± 0.7 (3)
69/55	GLN	CAG	ARG	19	CGG	1.4 ± 1.1 (3)
69/55	GLN	CAG	GLY	19	GGG	0.68 ± 0.02 (2)
69/55	GLN	CAG	LEU	19	CTC	-

[10] Double mutant; has Pro at position 73.

[11] Double mutant; has Thr at position 64.

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
70/56	ASN	AA(C/T)	LEU	19	TTG	0.032 (1)
70/56	ASN	AA(C/T)	VAL	19	GTG	-
70/56	ASN	AA(C/T)	TRP	19	TGG	-
70/56	ASN	AA(C/T)	PRO[12]	19	CCG	0.43 ± 0.29 (2)
70/56	ASN	AA(C/T)	ALA[13]	19	GCC	0.03 (1)
71/57	ALA	GCA	MET	19	ATG	0.23 (1)
71/57	ALA	GCA	LEU	19	CTG	<0.005 (1)
71/57	ALA	GCA	PRO	19	CCC	0.58 (1)
71/57	ALA	GCA	ARG	19	AGG	0.66 (1)
71/57	ALA	GCA	GLU	19	GAG	0.46 ± 0.27
71/57	ALA	GCA	THR	19	ACC	0.34 ± 0.41 (3)
71/57	ALA	GCA	GLN	19	GGC	0.42 ± 0.32 (3)
71/57	ALA	GCA	TRP	19	TGG	-
71/57	ALA	GCA	ASN	19	AAC	0.09 (1)
72/58	SER	TCA	GLU	19	GAG	0.62 ± 0.27 (3)
72/58	SER	TCA	MET	19	ATG	0.45 ± 0.55 (3)
72/58	SER	TCA	ALA	19	GCC	0.48 ± 0.33 (3)
72/58	SER	TCA	HIS	19	CAC	0.10 (1)
72/58	SER	TCA	ASN	19	AAC	0.38 ± 0.44 (3)
72/58	SER	TCA	ARG	19	CGG	0.81 ± 0.43 (4)
72/58	SER	TCA	ASP	19	GAC	0.58 ± 0.39 (3)
73/59	ALA	GCA	GLU	19	GAG	0.49 ± 0.32 (3)
73/59	ALA	GCA	ASP	19	GAC	0.27 (1)
73/59	ALA	GCA	LEU	19	CTG	0.55 ± 0.45 (4)
73/59	ALA	GCA	SER	19	AGC	0.37 ± 0.36 (2)
73/59	ALA	GCA	GLY	19	GGG	0.38 ± 0.32 (3)
73/59	ALA	GCA	THR	19	ACC	0.31 (1)
73/59	ALA	GCA	ARG	19	AGG	0.40 ± 0.18 (3)
74/60	ILE	AT(T/C)	MET	19	ATG	<0.16 (1)
74/60	ILE	AT(T/C)	THR	19	ACG	-

[12] Double mutant; has Pro at position 73.

[13] Double mutant; has Met at position 74.

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
74/60	ILE	AT(T/C)	PRO	19	CCG	-
74/60	ILE	AT(T/C)	ARG	19	AGG	-
74/60	ILE	AT(T/C)	GLY	19	GGG	0.006 (1)
74/60	ILE	AT(T/C)	ALA	19	GCG	-
75/61	GLU	GAG	LYS	19	AAG	0.07 ± 0.07 (2)
75/61	GLU	GAG	GLY	19	GGG	0.27 ± 0.20 (2)
75/61	GLU	GAG	ASP	19	GAC	0.18 (1)
75/61	GLU	GAG	PRO	19	CCG	-
75/61	GLU	GAG	TRP	19	TGG	-
75/61	GLU	GAG	ARG	19	CGG	-
75/61	GLU	GAG	SER	19	TCG	0.27 ± 0.22 (3)
75/61	GLU	GAG	GLN	19	CAG	0.40 ± 0.38 (3)
75/61	GLU	GAG	LEU	19	TTG	-
76/62	SER	AGC	VAL	19	GTG	1.0 ± 0.2 (2)
76/62	SER	AGC	ALA	19	GCG	0.94 ± 0.46 (2)
76/62	SER	AGC	ASN	19	AAC	1.2 (1)
76/62	SER	AGC	TRP	19	TGG	-
76/62	SER	AGC	GLU	19	GAG	0.90 ± 0.19 (2)
76/62	SER	AGC	PRO	19	CCG	2.1 ± 0.8 (4)
76/62	SER	AGC	GLY	19	GGC	1.3 ± 1.0 (4)
76/62	SER	AGC	ASP	19	GAC	0.29 (1)
77/63	ILE	ATT	SER	19	AGC	0.48 ± 0.38 (4)
77/63	ILE	ATT	ARG	19	CGC	0.09 ± 0.04 (2)
77/63	ILE	ATT	THR	19	ACG	<0.008 (1)
77/63	ILE	ATT	LEU	19	TTG	2.0 ± 0.1 (3)
78/64	LEU	CTT	ALA	19	GCG	-
78/64	LEU	CTT	SER	19	TCC	-
78/64	LEU	CTT	GLU	19	GAG	<0.006 (1)
78/64	LEU	CTT	PHE	19	TTC	-
78/64	LEU	CTT	GLY	19	GGG	-
78/64	LEU	CTT	ARG	19	AGG	-
79/65	LYS	AA(A/G)	THR	19	ACA	0.77 ± 0.91 (6)
79/65	LYS	AA(A/G)	GLY	19	GGG	1.1 ± 0.9 (6)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
79/65	LYS	AA(A/G)	ASN	19	AAC	1.0 ± 0.6 (6)
79/65	LYS	AA(A/G)	MET	19	ATG	1.6 ± 0.7 (6)
79/65	LYS	AA(A/G)	ARG	19	CGC	1.04 ± 0.7 (7)
79/65	LYS	AA(A/G)	ILE	19	ATC	1.0 ± 0.6 (6)
79/65	LYS	AA(A/G)	GLY	19	GGG	1.2 ± 0.4 (6)
79/65	LYS	AA(A/G)	ASP	19	GAC	0.72 ± 0.38 (7)
80/66	ASN	AAT	TRP	19	TGG	-
80/66	ASN	AAT	VAL	19	GTC	0.32 (1)
80/66	ASN	AAT	GLY	19	GGC	1.5 ± 1.4 (4)
80/66	ASN	AAT	THR	19	ACG	0.13 (1)
80/66	ASN	AAT	LEU	19	CTG	0.33 ± 0.14 (2)
80/66	ASN	AAT	GLU	19	GAG	1.1 ± 0.8 (4)
80/66	ASN	AAT	ARG	19	AGG	1.0 ± 0.8 (4)
81/67	LEU	CTC	GLN	19	CAA	-
81/67	LEU	CTC	GLY	19	GGC	<0.023 (1)
81/67	LEU	CTC	ALA	19	GCG	<0.047 (1)
81/67	LEU	CTC	TRP	19	TGG	<0.005 (1)
81/67	LEU	CTC	ARG	19	CGG	-
81/67	LEU	CTC	VAL	19	GTG	0.16 ± 0.18 (2)
81/67	LEU	CTC	LYS	19	AAG	-
82/68	LEU	C(TG/CC)	GLN	19	CAG	1.8 ± 0.3 (3)
82/68	LEU	C(TG/CC)	LYS	19	AAG	0.05 (1)
82/68	LEU	C(TG/CC)	TRP	19	TGG	2.7 ± 1.3 (4)
82/68	LEU	C(TG/CC)	ARG	19	AGC	1.1 ± 0.2 (3)
82/68	LEU	C(TG/CC)	ASP	19	GAC	2.7 ± 1.3 (4)
82/68	LEU	C(TG/CC)	VAL	19	GTG	1.5 ± 1.1 (5)
83/69	PRO	CCA	ALA	19	GCA	0.41 (1)
83/69	PRO	CCA	THR	19	ACC	0.66 ± 0.12 (3)
83/69	PRO	CCA	ARG	19	CGG	-
83/69	PRO	CCA	TRP	19	TGG	0.29 (1)
83/69	PRO	CCA	MET	19	ATG	0.43 ± 0.28 (3)
84/70	CYS	TG(T/C)	GLU	19	GAG	<0.014 (1)
84/70	CYS	TG(T/C)	GLY	19	GGG	<0.006 (1)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
84/70	CYS	TG(T/C)	ARG	19	AGG	-
84/70	CYS	TG(T/C)	MET	19	ATG	-
84/70	CYS	TG(T/C)	VAL	19	GTG	-
85/71	LEU	CTG	ASN	19	AAC	-
85/71	LEU	CTG	VAL	19	GTG	0.52 ± 0.21 (5)
85/71	LEU	CTG	GLN	19	CAG	-
86/72	PRO	CCC	CYS	19	TGC	-
86/72	PRO	CCC	ARG	19	AGG	-
86/72	PRO	CCC	ALA	19	GCG	-
86/72	PRO	CCC	LYS	19	AAG	-
87/73	LEU	(C/A)TG	SER	19	AGC	1.5 ± 0.4 (3)
87/73	LEU	(C/A)TG	TRP	19	TGG	-
87/73	LEU	(C/A)TG	GLY	19	GGG	-
88/74	ALA	GCC	LYS	19	AAG	-
88/74	ALA	GCC	ARG	19	AGG	0.11 ± 0.10 (2)
88/74	ALA	GCC	VAL	19	GTG	0.09 ±0.02 (2)
88/74	ALA	GCC	TRP	19	TGG	1.8 ± 0.2 (2)
89/75	THR	AC(G/A)	ASP	19	GAC	0.24 ± 0.10 (2)
89/75	THR	AC(G/A)	CYS	19	TGC	-
89/75	THR	AC(G/A)	LEU	19	CTC	0.01 (1)
89/75	THR	AC(G/A)	VAL	19	GTG	0.08 (1)
89/75	THR	AC(G/A)	GLU	19	GAG	0.11 (1)
89/75	THR	AC(G/A)	HIS	19	CAC	0.16 ± 0.06 (2)
89/75	THR	AC(G/A)	ASN	19	AAC	0.21 ± 0.04 (2)
89/75	THR	AC(G/A)	SER	19	TCG	0.25 ± 0.07 (2)
90/76	ALA	GCC	PRO	19	CCC	0.03 (1)
90/76	ALA	GCC	SER	19	TCG	-
90/76	ALA	GCC	THR	19	ACC	0.48 (1)
90/76	ALA	GCC	GLY	19	GGC	<0.006 (1)
90/76	ALA	GCC	ASP	19	GAC	0.44 ± 0.29 (4)
90/76	ALA	GCC	ILE	19	ATC	-
90/76	ALA	GCC	MET	19	ATG	0.25 ± 0.13 (2)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
91/77	ALA	GCA	PRO	19	CCC	1.9 ± 1.2 (3)
91/77	ALA	GCA	SER	19	TCC	0.12 ± 0.07 (2)
91/77	ALA	GCA	THR	19	ACC	0.48 ± 0.16 (2)
91/77	ALA	GCA	PHE	19	TTC	0.44 ± 0.50 (3)
91/77	ALA	GCA	LEU	19	CTC	0.43 ± 0.27 (5)
91/77	ALA	GCA	ASP	19	GAC	0.55 ± 0.09 (2)
91/77	ALA	GCA	HIS	19	CAC	-
92/78	PRO	CCC	PHE	19	TTC	-
92/78	PRO	CCC	ARG	19	CGG	-
92/78	PRO	CCC	SER	19	AGC	0.26 (1)
92/78	PRO	CCC	LYS	19	AAG	-
92/78	PRO	CCC	HIS	19	CAC	-
92/78	PRO	CCC	LEU	19	CTG	-
93/79	THR	ACG	ASP	19	GAC	1.3 ± 0.7 (4)
93/79	THR	ACG	SER	19	TCG	0.70 ± 0.56 (4)
93/79	THR	ACG	ASN	19	AAC	-
93/79	THR	ACG	PRO	19	CCC	0.53 ± 0.36 (4)
93/79	THR	ACG	ALA	19	GCG	1.13 ± 0.2 (3)
93/79	THR	ACG	LEU	19	CTG	0.69 ± 0.42
93/79	THR	ACG	ARG	19	CGC	0.93 ± 0.96 (4)
94/80	ARG	CGA	ILE	19	ATC	<0.020 (1)
94/80	ARG	CGA	SER	19	TCC	<0.100 (1)
94/80	ARG	CGA	GLU	19	GAG	<0.020 (1)
94/80	ARG	CGA	LEU	19	CTG	<0.020 (1)
94/80	ARG	CGA	VAL	19	GTG	<0.024 (1)
94/80	ARG	CGA	PRO	19	CCC	<0.024 (1)
95/81	HIS	CAT	GLN	19	CAG	<0.010 (1)
95/81	HIS	CAT	PRO	19	CCG	1.6 ± 0.8 (3)
95/81	HIS	CAT	ARG	19	CGC	4.7 ± 5.9 (2)
95/81	HIS	CAT	VAL	19	GTG	1.2 ± 1.7 (2)
95/81	HIS	CAT	LEU	19	CTC	0.7 (1)
95/81	HIS	CAT	GLY	19	GGC	1.7 ± 2.4 (5)
95/81	HIS	CAT	THR	19	ACC	2.9 ± 4.5 (4)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
95/81	HIS	CAT	TYR	19	TAC	0.07 (1)
96/82	PRO	CCA	LYS	19	AAG	<0.010 ±0.001(2)
96/82	PRO	CCA	TYR	19	TAC	0.69 (1)
96/82	PRO	CCA	GLY	19	GGG	<0.040 (1)
96/82	PRO	CCA	ILE	19	ATC	<0.040 (1)
96/82	PRO	CCA	THR	19	ACC	<0.040 (1)
97/83	ILE	ATC	VAL	19	GTC	0.91 ± 1.2 (8)
97/83	ILE	ATC	LYS	19	AAG	<0.024
97/83	ILE	ATC	ALA	19	GCG	0.15 (1)
97/83	ILE	ATC	ASN	19	AAT	<0.02 (1)
98/84	HIS	CAT	ILE	19	ATC	5.0 ± 4.9 (12)
98/84	HIS	CAT	ASN	19	AAC	1.4 ± 0.4 (2)
98/84	HIS	CAT	LEU	19	CTC	2.4 ± 1.0 (2)
98/84	HIS	CAT	ASP	19	GAC	0.38 ± 0.49 (5)
98/84	HIS	CAT	ALA	19	GCC	2.0 ± 1.0 (3)
98/84	HIS	CAT	THR	19	ACG	1.6 ± 0.3 (2)
98/84	HIS	CAT	LEU	19	TTG	1.5 (1)
98/84	HIS	CAT	PRO	19	CCG	0.55 (1)
99/85	ILE	ATC	LEU	19	CTG	1.4 ± 1.4 (7)
99/85	ILE	ATC	ARG	19	CGC	<0.025 (1)
99/85	ILE	ATC	ASP	19	GAC	<0.025 (1)
99/85	ILE	ATC	VAL	19	GTC	0.51 ± 0.59 (3)
99/85	ILE	ATC	PRO	19	CCG	<0.025 (1)
99/85	ILE	ATC	GLN	19	CAG	<0.018 ±0.010(2)
99/85	ILE	ATC	GLY	19	GGG	<0.018 ±0.10 (2)
99/85	ILE	ATC	SER	19	TCG	<0.025 (1)
99/85	ILE	ATC	PHE	19	TTC	0.45 (1)
99/85	ILE	ATC	HIS	19	CAC	<0.025 (1)
100/86	LYS	AAG	TYR	19	TAC	0.03 (1)
100/86	LYS	AAG	LEU	19	TTG	0.33 ± 0.31 (3)
100/86	LYS	AAG	HIS	19	CAC	0.36 ± 0.22 (9)
100/86	LYS	AAG	ARG	19	AGC	4.7 ± 5.9 (4)
100/86	LYS	AAG	ILE	19	ATC	0.95 (1)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
100/86	LYS	AAG	SER	19	AGC	0.95 (1)
100/86	LYS	AAG	GLN	19	CAG	0.78 ± 0.80 (7)
100/86	LYS	AAG	PRO	19	CCG	0.70 (1)
101/87	ASP	GAC	PRO	19	CCC	2.3 ± 3.1 (4)
101/87	ASP	GAC	MET	19	ATG	1.8 ± 2.5 (6)
101/87	ASP	GAC	LYS	19	AAG	1.2 ± 1.7 (3)
101/87	ASP	GAC	HIS	19	CAC	2.5 (1)
101/87	ASP	GAC	THR	19	ACG	0.90 ± 0.77 (3)
101/87	ASP	GAC	TYR	19	TAC	0.59 (1)
101/87	ASP	GAC	VAL	19	GTC	0.42 (1)
101/87	ASP	GAC	TYR	19	TAC	1.0 ± 0.02 (2)
101/87	ASP	GAC	GLN	19	CAG	0.07 (1)
102/88	GLY	GGT	LEU	19	CTC	<0.015 ±0.007(2)
102/88	GLY	GGT	GLU	19	GAG	0.40 ± 0.07 (3)
102/88	GLY	GGT	LYS	19	AGG	0.16 ± 0.14 (2)
102/88	GLY	GGT	SER	19	TCC	0.29 (1)
102/88	GLY	GGT	TYR	19	TAC	0.04 (1)
102/88	GLY	GGT	PRO	19	CCC	<0.011 (1)
103/89	ASP	GAC	SER	19	TCC	0.02 (1)
104/90	TRP	TGG	VAL	19	GTG	0.11 ± 0.06 (5)
104/90	TRP	TGG	CYS	19	AGC	0.07 ± 0.03 (5)
104/90	TRP	TGG	TYR	19	TAC	0.34 ± 0.42 (5)
104/90	TRP	TGG	THR	19	ACC	0.04 ± 0.02 (2)
104/90	TRP	TGG	MET	19	ATG	0.14 (1)
104/90	TRP	TGG	PRO	19	CCC	0.02 ± 0.02 (2)
104/90	TRP	TGG	LEU	19	TTG	0.65 ± 1.0 (3)
104/90	TRP	TGG	GLN	19	CAG	0.008 (1)
104/90	TRP	TGG	LYS	19	AAG	-
104/90	TRP	TGG	GLY	19	GAG	-
104/90	TRP	TGG	ALA	19	GCC	-
104/90	TRP	TGG	PHE	19	TTC	-
104/90	TRP	TGG	GLY	19	GGC	-
105/91	ASN	AAT	PRO	19	CCG	4.8 ± 8.5 (5)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON[5]	BIOL ACTIVITY
105/91	ASN	AAT	ALA	19	GCC	0.65 ± 0.30 (3)
105/91	ASN	AAT	PHE	19	TTC	0.13 (1)
105/91	ASN	AAT	SER	19	TCC	1.9 ± 2.7 (5)
105/91	ASN	AAT	TRP	19	TGG	0.95 (1)
105/91	ASN	AAT	GLN	19	CAA	0.57 ± 0.52 (3)
105/91	ASN	AAT	TYR	19	TAC	0.66 ± 0.53 (4)
105/91	ASN	AAT	LEU	19	CTC	0.87 ± 0.79 (2)
105/91	ASN	AAT	LYS	19	AAG	0.70 (1)
105/91	ASN	AAT	ILE	19	ATC	1.0 (1)
105/91	ASN	AAT	ASP	19	GAC	1.0 ± 0.9 (4)
105/91	ASN	AAT	HIS	19	CAC	0.71 ± 0.48 (2)
106/92	GLU	GAA	SER	19	TCC	0.17 ± 0.21 (2)
106/92	GLU	GAA	ALA	19	GCG	0.235 ± 0.26 (2)
106/92	GLU	GAA	LYS	19	AAG	-
106/92	GLU	GAA	THR	19	ACC	-
106/92	GLU	GAA	ILE	19	ATC	-
106/92	GLU	GAA	GLY	19	GGC	0.70 ± 0.76 (4)
106/92	GLU	GAA	PRO	19	CCC	-
108/94	ARG	CGG	LYS	19	AAG	0.11 ± 0.03 (2)
108/94	ARG	CGG	ASP	19	GAC	-
108/94	ARG	CGG	LEU	19	TTG	0.01 (1)
108/94	ARG	CGG	THR	19	ACG	0.08 (1)
108/94	ARG	CGG	ILE	19	ATC	<0.01 (1)
108/94	ARG	CGG	PRO	19	CCC	-
109/95	ARG	AGG	THR	19	ACC	1.1 ± 0.2 (3)
109/95	ARG	AGG	PRO	19	CCC	-
109/95	ARG	AGG	GLU	19	GAG	1.1 ± 0.1 (3)
109/95	ARG	AGG	TYR	19	TAC	<0.006 (1)
109/95	ARG	AGG	LEU	19	CTC	1.2 ± 0.9 (4)
109/95	ARG	AGG	SER	19	TCG	1.7 ± 0.8 (4)
109/95	ARG	AGG	GLY	19	GGG	0.17 (1)
110/96	LYS	AAA	ALA	19	GCC	<0.08 (1)
110/96	LYS	AAA	ASN	19	AAC	-

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
110/96	LYS	AAA	THR	19	ACG	-
110/96	LYS	AAA	LEU	19	CTC	-
110/96	LYS	AAA	ARG	19	CGG	-
110/96	LYS	AAA	GLN	19	CAG	-
110/96	LYS	AAA	TRP	19	TGG	-
111/97	LEU	CTG	ILE	19	ATC	-
111/97	LEU	CTG	ARG	19	CGG	-
111/97	LEU	CTG	ASP	19	GAC	-
111/97	LEU	CTG	MET	19	ATG	-
112/98	THR	ACG	VAL	19	GTG	0.55 ± 0.44 (3)
112/98	THR	ACG	GLN	19	CAG	1.7 ± 1.0 (3)
112/98	THR	ACG	TYR	19	TAC	<0.018 (1)
112/98	THR	ACG	GLU	19	GAG	0.12 (1)
112/98	THR	ACG	HIS	19	CAC	0.25 ± 0.40 (3)
112/98	THR	ACG	SER	19	TCC	0.17 ± 0.15 (2)
112/98	THR	ACG	PHE	19	TTC	-
113/99	PHE	TTC	SER	19	AGC	-
113/99	PHE	TTC	CYS	19	TGC	-
113/99	PHE	TTC	HIS	19	CAC	<0.009 (1)
113/99	PHE	TTC	GLY	19	GGC	-
113/99	PHE	TTC	TRP	19	TGG	-
113/99	PHE	TTC	TYR	19	TAC	0.07 (1)
113/99	PHE	TTC	ASN	19	AAC	-
114/100	TYR	TAT	CYS	19	TGC	-
114/100	TYR	TAT	HIS	19	CAC	-
114/100	TYR	TAT	SER	19	AGC	-
114/100	TYR	TAT	TRP	19	TGG	0.88 (1)
114/100	TYR	TAT	ARG	19	AGG	-
114/100	TYR	TAT	LEU	19	CTC	<0.018 (1)
115/101	LEU	CTG	ASN	19	AAC	<0.004 (1)
115/101	LEU	CTG	VAL	19	GTG	-
115/101	LEU	CTG	PRO	19	CCC	<0.004 (1)
115/101	LEU	CTG	ARG	19	AGG	<0.004 (1)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
115/101	LEU	CTG	ALA	19	GCG	0.50 (1)
115/101	LEU	CTG	HIS	19	CAC	-
115/101	LEU	CTG	THR	19	ACC	-
115/101	LEU	CTG	TRP	19	TGG	-
115/101	LEU	CTG	MET	19	ATG	<0.008 (1)
116/102	LYS	AAA	LEU[14]	19	TTG	-
116/102	LYS	AAA	PRO[14]	19	CCG	<0.004 (1)
116/102	LYS	AAA	THR[14]	19	ACC	0.50 (1)
116/102	LYS	AAA	MET[14]	19	ATG	0.13 (1)
116/102	LYS	AAA	ASP[14]	19	GAC	<0.018 (1)
116/102	LYS	AAA	VAL	19	GTG	2.3 ± 1.2 (5)
116/102	LYS	AAA	GLU	19	GAG	0.06 (1)
116/102	LYS	AAA	ARG	19	CGC	0.06 (1)
116/102	LYS	AAA	TRP	19	TGG	2.3 ± 1.0 (4)
116/102	LYS	AAA	SER	19	TCG	0.69 ± 0.51 (5)
116/102	LYS	AAA	LEU	19	CTC	0.14 ± 0.02 (2)
116/102	LYS	AAA	ILE	19	ATC	1.3 ± 0.3 (3)
116/102	LYS	AAA	THR	19	ACG	0.84 ± 0.30 (4)
117/103	THR	ACC	SER	19	AGC	1.1 ± 0.2 (3)
117/103	THR	ACC	ASN	19	AAC	0.31 ± 0.39 (3)
117/103	THR	ACC	ILE	19	ATC	-
117/103	THR	ACC	TRP	19	TGG	0.02 (1)
117/103	THR	ACC	LYS	19	AAG	<0.005 (1)
117/103	THR	ACC	PRO	19	CCG	-
118/104	LEU	CTT	SER	19	TCA	-
118/104	LEU	CTT	PRO	19	CCC	-
118/104	LEU	CTT	ALA	19	GCC	-
118/104	LEU	CTT	GLU	19	GAG	-
118/104	LEU	CTT	CYS	19	TGC	-
118/104	LEU	CTT	ASP	19	GAC	-
118/104	LEU	CTT	TYR	19	TAC	-

[14] Double mutant: has Ser at position 105.

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
119/105	GLU	GAG	SER	19	TCC	0.26 ± 0.19 (2)
119/105	GLU	GAG	LYS	19	AAG	0.04 (1)
119/105	GLU	GAG	PRO	19	CCG	0.31 ± 0.27 (3)
119/105	GLU	GAG	LEU	19	CTG	0.35 ± 0.35 (3)
119/105	GLU	GAG	THR	19	ACC	0.25 ± 0.27 (3)
119/105	GLU	GAG	TYR	19	TAC	0.30 ± 0.32 (3)
119/105	GLU	GAG	ARG	19	CGC	0.06 (1)
120/106	ASN	AAT	ALA	19	GCC	<0.009 (1)
120/106	ASN	AAT	PRO	19	CCC	1.7 ± 0.7 (3)
120/106	ASN	AAT	LEU	19	TTG	1.2 ± 0.3 (3)
120/106	ASN	AAT	HIS	19	CAC	1.0 ± 0.3 (2)
120/106	ASN	AAT	VAL	19	GTG	1.7 ± 0.3 (3)
120/106	ASN	AAT	GLN	19	CAG	0.85 ± 0.16 (2)
121/107	ALA	GCG	SER	19	AGC	1.2 ± 0.2 (3)
121/107	ALA	GCG	ILE	19	ATC	2.8 ± 2.5 (2)
121/107	ALA	GCG	ASN	19	AAC	0.91 ± 0.77 (5)
121/107	ALA	GCG	PRO	19	CCG	1.3 (1)
121/107	ALA	GCG	LYS	19	AAG	0.26 ± 0.24 (2)
121/107	ALA	GCG	ASP	19	GAC	1.8* ± 0.9 (3)
121/107	ALA	GCG	GLY	19	GGC	0.69 (1)
122/108	GLN	GCG	SER	19	AGC	0.96 ± 0.41 (3)
122/108	GLN	CA(G/A)	MET	19	ATG	1.7 ± 0.5 (3)
122/108	GLN	CA(G/A)	TRP	19	TGG	1.4 (1)
122/108	GLN	CA(G/A)	ARG	19	AGG	0.78 (1)
122/108	GLN	CA(G/A)	PHE	19	TTC	2.3 ± 1.1 (3)
122/108	GLN	CA(G/A)	PRO	19	CCG	1.0 (1)
122/108	GLN	CA(G/A)	HIS	19	CAC	1.4 (1)
122/108	GLN	CA(G/A)	ILE	19	ATC	2.7 ± 0.8 (3)
122/108	GLN	CA(G/A)	TYR	19	TAC	1.7 ± 0.3 (2)
122/108	GLN	CA(G/A)	CYS	19	TGC	0.58 (1)
123/109	ALA	GCT	MET	19	ATG	2.0 ± 0.2 (3)
123/109	ALA	GCT	GLU	19	GAG	2.1 ± 1.0 (3)
123/109	ALA	GCT	HIS	19	CAC	0.98 ± 0.72 (3)

	PARENTAL		(15-125)hIL-3 MUTANT			
hIL-3 aa POSITION[1]	aa	CODON	aa	SEQ ID NO:	CODON	BIOL ACTIVITY
123/109	ALA	GCT	SER	19	AGC	1.4 ± 0.8 (3)
123/109	ALA	GCT	PRO	19	CCC	0.64 ± 0.16 (2)
123/109	ALA	GCT	TYR	19	TAC	0.51 ± 0.25 (2)
123/109	ALA	GCT	LEU	19	CTG	1.2 ± 0.1 (2)

The mutants in Table 6 were made as described in the Examples, particularly Examples 19, 20, 21 and 38 to 53.

It will be apparent to those skilled in the art that other codons besides those shown in Table 6 can also code for the substituted amino acids in the hIL-3 muteins. The present invention includes the DNAs encoding the mutant hIL-3 polypeptides of the invention including the various codons which can code for the parental and substituted amino acids of the hIL-3 muteins of the invention due to the degeneracy of the genetic code.

hIL-3 (15-125) variant genes encoding the variants listed in Table 6 can also be expressed from intracellular expression vectors to produce large quantities of the variant protein which can be purified and assayed for biological activity. The hIL-3 variant genes, from Table 6, can be excised from the secretion expression vector, as a 345 base pair NcoI/HindIII fragment and ligated into an appropriate intracellular expression vector, such as pMON2341 digested with NcoI and HindIII. Examples of variants transferred to pMON2341 in this manner are shown in Table 7. Two examples of such a transfer are described in the construction of pMON13215 (EXAMPLE 64) and pMON13252 (EXAMPLE 65).

EXAMPLE 64

Construction of pMON13215

Plasmid, pMON2341, DNA was digested with restriction enzymes NcoI and HindIII resulting in a 3619 base pair NcoI/HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, F1 phage origin of replication as the transcription terminator, precA, g10L ribosome binding site. The plasmid encoding the hIL-3 (15-125) $Trp^{(116)}$ variant, from Table 6 was digested with NcoI and HindIII resulting in a 345 base pair NcoI/HindIII fragment. The 345 Base pair NcoI/HindIII fragment was ligated with the 3619 base pair fragment from pMON2341 and the ligation reaction mixture was used to transform E.coli K-12 strain JM101. Plasmid DNA was isolated and screened by restriction anaylsis using NcoI and HindIII. Positive clones contained a 345 base pair NcoI/HindIII. This construct was designated PMON13215. The plasmid, pMON13215, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

PEPTIDE A9; (15-125)HIL-3 $TRP^{(116)}$ PMON13215

```
          Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
          15                  20                  25
      Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
          30                  35                  40
      Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
          45                  50                  55
      Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
          60                  65                  70
      Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
          75                  80                  85
      Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
          90                  95                  100
```

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Trp Thr
105 110 115
Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:217]
120 125

DNA sequence #A9 pMON13215 116w

ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA
GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGGT GAAGACCAAG
ATATCCTGAT GGAAAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC
CGTGCTGTCA ACTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA
AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC
CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC
TTCTATCTGT GGACCTTGGA GAACGCGCAG GCTCAACAG
[SEQ ID NO:220]

EXAMPLE 65

Construction of pMON13252

Plasmid, pMON2341, DNA was digested with restriction enzymes NcoI and HindIII resulting in a 3619 base pair NcoI/HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication F1 phage origin of replication as the transcription terminator, precA, g10L ribosome binding site. The plasmid encoding the hIL-3 (15-125) $Asp^{(50)}$ variant, from Table 6, was digested with NcoI and HindIII resulting in a 345 base pair NcoI/HindIII fragment. This 345 Base pair NcoI/HindIII fragment was ligated with the 3619 base pair fragment from pMON2341 and the ligation reaction mixture was used to transform E.coli K-12 strain JM101. Plasmid DNA was isolated and screened by restriction analysis using NcoI and HindIII. Positive clones contained a 345 base pair NcoI/HindIII. This construct was designated pMON13252. The plasmid, pMON13252, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

PEPTIDE A10; (15-125)HIL-3 ASP$^{(50)}$ pMON13252

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
15 20 25
Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
30 35 40
Glu Asp Gln Asp Ile Leu Met Asp Asn Asn Leu Arg Arg Pro Asn
45 50 55
Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
60 65 70
Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
75 80 85
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
90 95 100
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
105 110 115
Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:218]
120 125

DNA sequence #A10 pMON13252 50D

ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA
GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGGT GAAGACCAAG
ATATCCTGAT GGAAAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC
CGTGCTGTCA ACTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA
AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC
CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC
TTCTATCTGA AAACCTTGGA GAACGCGCAG GCTCAACAG
[SEQ ID NO:216]

Table7

pMON number	position/ mutant (15-125) hIL-3	NATIVE amino acid	SUBSTITUTION amino acid	SEQ ID NO:	RELATIVE POTENCY
pMON13201	45/31M	Gln	Met	19	6.3
pMON13202	51/37R	Asn	Arg	19	1.58
pMON13203	51/37P	Asn	Pro	19	2.5
pMON13204	51/37T	Asn	Thr	19	3.16
pMON13205	56/42S	Pro	Ser	19	6.3
pMON13206	98/84I	His	Ile	19	6.3
pMON13207	45/31V	Gln	Val	19	4
pMON13208	42/28D	Gly	Asp	19	6.3
pMON13209	42/28S	Gly	Ser	19	12.6
pMON13210	42/28A	Gly	Ala	19	2.5
pMON13211	46/32S	Asp	Ser	19	16
pMON13212	82/68W	Leu	Trp	19	5
pMON13213	82/68D	Leu	Asp	19	4
pMON13214	100/86R	Lys	Arg	19	4
pMON13215	116/102W	Lys	Trp	19	31
pMON13216	23/9L	Ile	Leu	19	4
pMON13217	32/18R	Leu	Arg	19	7.9
pMON13218	32/18N	Leu	Asn	19	2
pMON13219	32/18A	Leu	Ala	19	1.58
pMON13220	34/20S	Leu	Ser	19	6.3
pMON13221	34/20M	Leu	Met	19	6.3
pMON13222	50/36D	Glu	Asp	19	7.9
pMON13223	62/48I	Asn	Ile	19	*
pMON13224	66/52R	Lys	Arg	19	4
pMON13225	76/62P	Ser	Pro	19	1.25
pMON13226	77/63L	Ile	Leu	19	1.58
pMON13227	22/8G	Glu	Gly	19	0.008
pMON13228	115/101M	Leu	Met	19	0.04
pMON13229	122/108I	Gln	Ile	19	1
pMON13231	51/37H	Asn	His	19	1.25
pMON13232	59/45L	Glu	Leu	19	1.99
pMON13233	63/49H	Arg	His	19	*
pMON13234	64/50N	Ala	Asn	19	0.03
pMON13235	65/51T	Val	Thr	19	1.58
pMON13236	76/62V	Ser	Val	19	2.5
pMON13237	76/62A	Ser	Ala	19	5
pMON13238	91/77P	Ala	Pro	19	*
pMON13240	100/86Q	Lys	Gln	19	2.5
pMON13241	101/87M	Asp	Met	19	6.3
pMON13242	105/91N	Asn	Asn	19	*
pMON13243	116/102V	Lys	Val	19	7.9

Table7

	position/				
	mutant	Native	SUBSTITUTION	SEQ ID NO:	RELATIVE
pMON number	(15-125) hIL-3	amino acid	amino acid		POTENCY
pMON13244	122/108F	Gln	Phe	19	6.3
pMON13245	123/109E	Ala	Glu	19	1.58
	position/				
	mutant	NATIVE	SUBSTITUTION	SEQ ID NO:	RELATIVE
pMON number	(1-133) hIL-3	amino acid	amino acid		POTENCY
pMON13246	42D	Gly	Asp	15	20
pMON13247	42S	Gly	Ser	15	*
pMON13248	42A	Gly	Ala	15	16
pMON13249	45V	Gln	Val	15	5
pMON13250	45M	Gln	Met	15	*
pMON13251	46S	Asp	Ser	15	5
pMON13252	50D	Glu	Asp	15	5
pMON13253	98I	His	Ile	15	*
pMON13264	97V	Ile	Val	15	4
pMON13266	75K	Glu	Lys	15	0.25
pMON13267	89N	Thr	Asn	15	2.5

Table 7 shows the biological activity of (15-125)hIL-3 mutant polypeptides of the present invention expressed from intracellular expression vectors. Upon expression these muteins may have Met- or Met-Ala- preceding the initial (15-125)hIL-3 amino acid. The relative biological activity of IL-3 mutants is calculated by dividing the EC_{50} (1-133) hIL-3 by the EC_{50} of the mutant.

Example 66

The variants in Table 8 were constructed by cassette mutagenesis using methods described in the Materials and Methods and the Examples contained herein, particularly Examples 54-57. Parental plasmid DNA (Table 8), digested with the appropriate restriction enzymes (Table 8), was ligated with the indicated annealed pairs of complementary oligonucleotides (Table 8). The assembled oligonucleotides create appropriate restriction ends and a portion of the (15-125) hIL-3 gene sequence Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made. The oligonucleotides create change(s) in the (15-125) hIL-3 gene which enclode the corresponding amino acid substitution in the variant polypeptide (Table 8). The amino acids substitutions in polypeptide #1 (SEQ ID NO:65) are indicated in Table 8.

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
21 asp	glu	GAA	21glu1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:73	SEQ ID NO:523	SEQ ID NO:524				
			21glu4	NcoRV5	NcoRV6				
			SEQ ID NO:219	SEQ ID NO:526	SEQ ID NO:527				
21 asp	gln	CAA	21gln1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:71	SEQ ID NO:523	SEQ ID NO:524				
			21gln4	NcoRV5	NcoRV6				
			SEQ ID NO:72	SEQ ID NO:526	SEQ ID NO:527				
21 asp	asn	AAC	21asn1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:68	SEQ ID NO:523	SEQ ID NO:524				
			21asn4	NcoRV5	NcoRV6				
			SEQ ID NO:70	SEQ ID NO:526	SEQ ID NO:527				
21 asp	thr	ACC	21thr1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:232	SEQ ID NO:523	SEQ ID NO:524				
			21thr4	NcoRV5	NcoRV6				
			SEQ ID NO:233	SEQ ID NO:526	SEQ ID NO:527				
21 asp	ser	AGC	21ser1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:230	SEQ ID NO:523	SEQ ID NO:524				
			21ser4	NcoRV5	NcoRV6				
			SEQ ID NO:231	SEQ ID NO:526	SEQ ID NO:527				
22 glu	asp	GAC	22asp1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:236	SEQ ID NO:523	SEQ ID NO:524				
			22asp4	NcoRV5	NcoRV6				
			SEQ ID NO:237	SEQ ID NO:526	SEQ ID NO:527				
22 glu	asn	AAC	22asn1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:234	SEQ ID NO:523	SEQ ID NO:524				
			22asn4	NcoRV5	NcoRV6				
			SEQ ID NO:235	SEQ ID NO:526	SEQ ID NO:527				
22 glu	gln	CAG	22gln1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:238	SEQ ID NO:523	SEQ ID NO:524				
			22gln4	NcoRV5	NcoRV6				
			SEQ ID NO:239	SEQ ID NO:526	SEQ ID NO:527				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
22 glu	leu	CTG	22leu1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:240	SEQ ID NO:523	SEQ ID NO:524				
			22leu4	NcoRV5	NcoRV6				
			SEQ ID NO:241	SEQ ID NO:526	SEQ ID NO:527				
22 glu	val	GTT	22val1	NcoRv2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:242	SEQ ID NO:523	SEQ ID NO:524				
			22val4	NcoRV5	NcoRV6				
			SEQ ID NO:243	SEQ ID NO:526	SEQ ID NO:527				
34 leu	glu	GAA	NcoRV1	34Glu2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO: 251	SEQ ID NO:524				
			NcoRV4	34Glu5	NcoRV6				
			SEQ ID NO:525	SEQ ID NO: 252	SEQ ID NO:527				
34 leu	gln	CAG	NcoRV1	34gln2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:248	SEQ ID NO:524				
			NcoRV4	34gln5	NcoRV6				
			SEQ ID NO:525	SEQ ID NO:249	SEQ ID NO:527				
34 leu	thr	ACC	NcoRV1	34thr2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:256	SEQ ID NO:524				
			NcoRV4	34thr5	NcoRV6				
			SEQ ID NO:525	SEQ ID NO:257	SEQ ID NO:527				
34 leu	arg	CGT	NcoRV1	34arg2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:246	SEQ ID NO:524				
			NcoRV4	34arg5	NcoRV6				
			SEQ ID NO:525	SEQ ID NO:247	SEQ ID NO:527				
34 leu	ala	GCT	NcoRV1	34ala2	NcoRV3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:244	SEQ ID NO:524				
			NcoRV4	34ala5	NcoRV6				
			SEQ ID NO:525	SEQ ID NO:245	SEQ ID NO:527				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
34 leu	phe	TTC	NaoRV1	34phe2	NaoRV3			pMON13356	NaoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:254	SEQ ID NO:524				
			NaoRV4	34phe5	NaoRV6				
			SEQ ID NO:525	SEQ ID NO:255	SEQ ID NO:527				
34 leu	ile	ATC	NaoRV1	34ile2	NaoRV3			pMON13356	NaoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:252	SEQ ID NO:524				
			NaoRV4	34ile5	NaoRV6				
			SEQ ID NO:525	SEQ ID NO:253	SEQ ID NO:527				
42 gly	lys	AAA	NaoRV1	NaoRv2	42lys3			pMON13356	NaoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:268				
			NaoRV4	NaoRV5	42lys6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:269				
42 gly	asn	AAC	NaoRV1	NaoRv2	42asn3			pMON13356	NaoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:260				
			NaoRV4	NaoRV5	42asn6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:261				
42 gly	thr	ACC	NaoRV1	NaoRv2	42thr3			pMON13356	NaoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:274				
			NaoRV4	NaoRV5	42thr6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:275				
42 gly	leu	CTG	NaoRV1	NaoRv2	42leu3			pMON13356	NaoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:266				
			NaoRV4	NaoRV5	42leu6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:267				
42 gly	val	GTT	NaoRV1	NaoRv2	42val3			pMON13356	NaoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:278				
			NaoRV4	NaoRV5	42val6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:279				
42 gly	glu	GAA	NaoRV1	NaoRv2	42glu3			pMON13356	NaoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:262				
			NaoRV4	NaoRV5	42glu6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:263				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
42 gly	phe	TTC	NcoRV1	NcoRv2	42phe3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:272				
			NcoRV4	NcoRV5	42phe6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:273				
42 gly	tyr	TAC	NcoRV1	NcoRv2	42tyr3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:276				
			NcoRV4	NcoRV5	42tyr6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:277				
42 gly	ile	ATC	NcoRV1	NcoRv2	42ile3			pMON13358	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:264				
			NcoRV4	NcoRV5	42ile6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:265				
42 gly	met	ATG	NcoRV1	NcoRv2	42met3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:270				
			NcoRV4	NcoRV5	42met6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:271				
43 glu	gln	CAG	NcoRV1	NcoRv2	43gln3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:282				
			NcoRV4	NcoRV5	43gln6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:283				
43 glu	arg	CGT	NcoRV1	NcoRv2	43arg3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:280				
			NcoRV4	NcoRV5	43arg6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:281				
43 glu	thr	ACC	NcoRV1	NcoRv2	43thr3			pMON13358	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:286				
			NcoRV4	NcoRV5	43thr6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:287				
43 glu	gly	GGT	NcoRV1	NcoRv2	43gly3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO284				
			NcoRV4	NcoRV5	43gly6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:285				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
44 asp	glu	GAA	NcoRV1	NcoRv2	44glu3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:294				
			NcoRV4	NcoRV5	44glu6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:295				
44 asp	asn	AAC	NcoRV1	NcoRv2	44asn3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:290				
			NcoRV4	NcoRV5	44asn6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:291				
44 asp	gln	CAG	NcoRV1	NcoRv2	44gln3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:292				
			NcoRV4	NcoRV5	44gln6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:293				
44 asp	ala	GCT	NcoRV1	NcoRv2	44ala3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:288				
			NcoRV4	NcoRV5	44ala6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:289				
45 gln	asp	GAC	NcoRV1	NcoRv2	45asp3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:302				
			NcoRV4	NcoRV5	45asp6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:303				
45 gln	asn	AAC	NcoRV1	NcoRv2	45asn3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:300				
			NcoRV4	NcoRV5	45asn6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:301				
45 gln	arg	CGT	NcoRV1	NcoRv2	45arg3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:298				
			NcoRV4	NcoRV5	45arg6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:299				
45 gln	ser	TCC	NcoRV1	NcoRv2	45ser3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:310				
			NcoRV4	NcoRV5	45ser6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:311				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
45 gln	ala	GCT	NcoRV1	NcoRv2	45ala3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:296				
			NcoRV4	NcoRV5	45ala6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:297				
45 gln	ile	ATC	NcoRV1	NcoRv2	45ile3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:308				
			NcoRV4	NcoRV5	45ile6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:309				
45 gln	glu	GAA	NcoRV1	NcoRv2	45glu3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:304				
			NcoRV4	NcoRV5	45glu6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:305				
45 gln	his	CAC	NcoRV1	NcoRv2	45his3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:306				
			NcoRV4	NcoRV5	45his6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:307				
46 asp	glu	GAA	NcoRV1	NcoRv2	46glu3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:318				
			NcoRV4	NcoRV5	46glu6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:319				
46 asp	asn	AAC	NcoRV1	NcoRv2	46asn3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:314				
			NcoRV4	NcoRV5	46asn6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:315				
46 asp	gln	CAG	NcoRV1	NcoRv2	46gln3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:316				
			NcoRV4	NcoRV5	46gln6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:317				
46 asp	lys	AAA	NcoRV1	NcoRv2	46lys3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:326				
			NcoRV4	NcoRV5	46lys6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:327				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
46 asp	his	CAC	NcoRV1	NcoRv2	46his3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:320				
			NcoRV4	NcoRV5	46his6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:321				
46 asp	ala	GCT	NcoRV1	NcoRv2	46ala3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:312				
			NcoRV4	NcoRV5	46ala6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:313				
46 asp	tyr	TAC	NcoRV1	NcoRv2	46tyr3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:328				
			NcoRV4	NcoRV5	46tyr6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:329				
46 asp	ile	ATC	NcoRV1	NcoRv2	46ile3			pMON13358	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:322				
			NcoRV4	NcoRV5	46ile6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:323				
46 asp	val	GTT	NcoRV1	NcoRv2	46val3			pMON13356	NcoI,EcoRV
			SEQ ID NO:522	SEQ ID NO:523	SEQ ID NO:330				
			NcoRV4	NcoRV5	46val6				
			SEQ ID NO:525	SEQ ID NO:526	SEQ ID NO:331				
48 leu	glu	GAA	48glu1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:334	SEQ ID NO:529	SEQ ID NO:530				
			48glu4	RVNsl5	RVNsl6				
			SEQ ID NO:535	SEQ ID NO:532	SEQ ID NO:533				
48 leu	lys	AAA	48lys1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:336	SEQ ID NO:529	SEQ ID NO:530				
			48lys4	RVNsl5	RVNsl6				
			SEQ ID NO:337	SEQ ID NO:532	SEQ ID NO:533				
48 leu	thr	ACC	48thr1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:340	SEQ ID NO:529	SEQ ID NO:530				
			48thr4	RVNsl5	RVNsl6				
			SEQ ID NO:341	SEQ ID NO:532	SEQ ID NO:533				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
48 leu	ala	GCT	48ala1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:332	SEQ ID NO:529	SEQ ID NO:530				
			48ala4	RVNsl5	RVNsl6				
			SEQ ID NO:333	SEQ ID NO:532	SEQ ID NO:533				
48 leu	met	ATG	48met1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:338	SEQ ID NO:529	SEQ ID NO:530				
			48met4	RVNsl5	RVNsl6				
			SEQ ID NO:339	SEQ ID NO:532	SEQ ID NO:533				
48 leu	val	CAC	48val1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:342	SEQ ID NO:529	SEQ ID NO:530				
			48val4	RVNsl5	RVNsl6				
			SEQ ID NO:343	SEQ ID NO:532	SEQ ID NO:533				
50 glu	lys	AAA	50lys1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:356	SEQ ID NO:529	SEQ ID NO:530				
			50lys4	RVNsl5	RVNsl6				
			SEQ ID NO:357	SEQ ID NO:532	SEQ ID NO:533				
50 glu	asn	AAC	50asn1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:352	SEQ ID NO:529	SEQ ID NO:530				
			50asn4	RVNsl5	RVNsl6				
			SEQ ID NO:353	SEQ ID NO:532	SEQ ID NO:533				
50 glu	ser	TCC	50ser1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:358	SEQ ID NO:529	SEQ ID NO:530				
			50ser4	RVNsl5	RVNsl6				
			SEQ ID NO:359	SEQ ID NO:532	SEQ ID NO:533				
50 glu	ala	GCT	50ala1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:350	SEQ ID NO:529	SEQ ID NO:530				
			50ala4	RVNsl5	RVNsl6				
			SEQ ID NO:351	SEQ ID NO:532	SEQ ID NO:533				
50 glu	ile	ATC	50ile1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:354	SEQ ID NO:529	SEQ ID NO:530				
			50ile4	RVNsl5	RVNsl6				
			SEQ ID NO:355	SEQ ID NO:532	SEQ ID NO:533				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
50 glu	val	GTT	50val1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:360	SEQ ID NO:529	SEQ ID NO:530				
			50val4	RVNsl5	RVNsl6				
			SEQ ID NO:361	SEQ ID NO:532	SEQ ID NO:533				
50 glu	his	CAC	50his1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:344	SEQ ID NO:529	SEQ ID NO:530				
			50his4	RVNsl5	RVNsl6				
			SEQ ID NO:345	SEQ ID NO:532	SEQ ID NO:533				
50 glu	phe	TTC	50phe1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:348	SEQ ID NO:529	SEQ ID NO:530				
			50phe4	RVNsl5	RVNsl6				
			SEQ ID NO:349	SEQ ID NO:532	SEQ ID NO:533				
50 glu	met	ATG	50met1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:346	SEQ ID NO:529	SEQ ID NO:530				
			50met4	RVNsl5	RVNsl6				
			SEQ ID NO:347	SEQ ID NO:532	SEQ ID NO:533				
54 arg	asn	AAC	54asn1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:364	SEQ ID NO:529	SEQ ID NO:530				
			54asn4	RVNsl5	RVNsl6				
			SEQ ID NO:365	SEQ ID NO:532	SEQ ID NO:533				
54 arg	lys	AAA	54lys1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:368	SEQ ID NO:529	SEQ ID NO:530				
			54lys4	RVNsl5	RVNsl6				
			SEQ ID NO:369	SEQ ID NO:532	SEQ ID NO:533				
54 arg	his	CAC	54his1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:366	SEQ ID NO:529	SEQ ID NO:530				
			54his4	RVNsl5	RVNsl6				
			SEQ ID NO:367	SEQ ID NO:532	SEQ ID NO:533				
54 arg	ala	GCT	54ala1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:362	SEQ ID NO:529	SEQ ID NO:530				
			54ala4	RVNsl5	RVNsl6				
			SEQ ID NO:363	SEQ ID NO:532	SEQ ID NO:533				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
56 pro	glu	GAA	56glu1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:376	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56glu5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:377	SEQ ID NO:533				
56 pro	gln		56gln1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:374	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56gln5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:375	SEQ ID NO:533				
56 pro	arg	CGT	56arg1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:372	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56arg5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:373	SEQ ID NO:533				
56 pro	his	CAC	56his1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:378	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56his5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:379	SEQ ID NO:533				
56 pro	thr	ACC	56thr1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:384	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56thr5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:385	SEQ ID NO:533				
56 pro	ala	GCT	56ala1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:370	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56ala5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:371	SEQ ID NO:533				
56 pro	tyr	TAC	56tyr1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:386	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56tyr5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:387	SEQ ID NO:533				
56 pro	phe	TTC	56phe1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:382	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56phe5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:383	SEQ ID NO:533				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
56 pro	leu	CTG	56leu1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:380	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56leu5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:381	SEQ ID NO:533				
56 pro	val	GTT	56val1	RVNsl2	RVNsl3			pMON13357	EcoRV,NcoI
			SEQ ID NO:388	SEQ ID NO:529	SEQ ID NO:530				
			RVNsl4	56val5	RVNsl6				
			SEQ ID NO:531	SEQ ID NO:389	SEQ ID NO:533				
82 leu	glu	GAA	NsiEco1	82glu2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:394	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82glu6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:395	SEQ ID NO:542	SEQ ID NO:545			
82 leu	asn	AAC	NsiEco1	82asn2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:392	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82asn6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:393	SEQ ID NO:542	SEQ ID NO:545			
82 leu	his	CAC	NsiEco1	82his2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:396	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82his6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:397	SEQ ID NO:542	SEQ ID NO:545			
82 leu	thr	ACC	NsiEco1	82thr2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:406	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82thr6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:407	SEQ ID NO:542	SEQ ID NO:545			
82 leu	ser	TCC	NsiEco1	82ser2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:404	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82ser6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:405	SEQ ID NO:542	SEQ ID NO:545			
82 leu	ala	GCT	NsiEco1	82ala2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:390	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82ala6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:391	SEQ ID NO:542	SEQ ID NO:545			

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
82 leu	tyr	TAC	NsiEco1	82tyr2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:408	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82tyr6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:409	SEQ ID NO:542	SEQ ID NO:545			
82 leu	phe	TTC	NsiEco1	82phe2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:402	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82phe6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:403	SEQ ID NO:542	SEQ ID NO:545			
82 leu	ile	ATC	NsiEco1	82ile2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:398	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82ile6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:399	SEQ ID NO:542	SEQ ID NO:545			
82 leu	met	ATG	NsiEco1	82met2	NsiEco3	NsiEco4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:400	SEQ ID NO:536	SEQ ID NO:539			
			NsiEco5	82met6	NsiEco7	NsiEco8			
			SEQ ID NO:540	SEQ ID NO:401	SEQ ID NO:542	SEQ ID NO:545			
92 pro	ala	GCT	NsiEco1	NsiEco2	92ala3A	NsiEco3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:410	SEQ ID NO:538	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	92ala7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:411	SEQ ID NO:545		
92 pro	gly	GGT	NsiEco1	NsiEco2	92gly3A	NsiEco3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:412	SEQ ID NO:538	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	92ala7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:413	SEQ ID NO:545		
92 pro	ile	ATC	NsiEco1	NsiEco2	92ile3A	NsiEco3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:414	SEQ ID NO:538	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	92ala7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:415	SEQ ID NO:545		
94 arg	gln	CAG	NsiEco1	NsiEco2	NsiEco3A	94gln3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:416	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	94gln7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:419	SEQ ID NO:545		

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
94 arg	lys	AAA	NsiEco1	NsiEco2	NsiEco3A	94lys3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:422	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	94lys7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:423	SEQ ID NO:545		
94 arg	his	CAC	NsiEco1	NsiEco2	NsiEco3A	94his3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:420	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	94his7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:538	SEQ ID NO:543	SEQ ID NO:421	SEQ ID NO:545		
94 arg	ala	GCT	NsiEco1	NsiEco2	NsiEco3A	94ala3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:416	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	94ala7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:417	SEQ ID NO:545		
95 his	asn	AAC	NsiEco1	NsiEco2	NsiEco3A	95asn3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:426	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	95asn7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:427	SEQ ID NO:545		
95 his	lys	AAA	NsiEco1	NsiEco2	NsiEco3A	95lys3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:432	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	95lys7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:433	SEQ ID NO:545		
95 his	ser	TCC	NsiEco1	NsiEco2	NsiEco3A	95ser3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:436	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	95ser7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:439	SEQ ID NO:545		
95 his	ala	GCT	NsiEco1	NsiEco2	NsiEco3A	95ala3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:424	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	95ala7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:425	SEQ ID NO:545		
95 his	trp	TGG	NsiEco1	NsiEco2	NsiEco3A	95trp3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:440	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	95trp7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:441	SEQ ID NO:545		

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
95 his	phe	TTC	NsiEco1	NsiEco2	NsiEco3A	95phe3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:11	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	95phe7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:437	SEQ ID NO:545		
95 his	ile	ATC	NsiEco1	NsiEco2	NsiEco3A	95ile3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:430	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	95ile7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:431	SEQ ID NO:545		
98 his	glu	GAA	NsiEco1	NsiEco2	NsiEco3A	98glu3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:446	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	98glu7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:447	SEQ ID NO:545		
98 his	gln	CAA	NsiEco1	NsiEco2	NsiEco3A	98gln3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:444	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	98gln7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:445	SEQ ID NO:545		
98 his	ser	TCC	NsiEco1	NsiEco2	NsiEco3A	98ser3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:452	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	98ser7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:453	SEQ ID NO:545		
98 his	phe	TTC	NsiEco1	NsiEco2	NsiEco3A	98phe3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:450	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	98phe7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:451	SEQ ID NO:545		
98 his	met	ATG	NsiEco1	NsiEco2	NsiEco3A	98met3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:448	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	98met7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:449	SEQ ID NO:545		
98 his	val	GTA	NsiEco1	NsiEco2	NsiEco3A	98val3B	NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:537	SEQ ID NO:454	SEQ ID NO:539		
			NsiEco5	NsiEco6	NsiEco7A	98val7B	NsiEco8		
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:543	SEQ ID NO:455	SEQ ID NO:545		

TABLE 8

position/native a.a.	substitution	codon	oligo pair		oligo pair		oligo pair		oligo pair		parental plasmid	restriction digest
98 his	lys	AAA	NsiEco1		NsiEco2		NsiEco3A		98lys3B		NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534		SEQ ID NO:535		SEQ ID NO:537		SEQ ID NO:458		SEQ ID NO:539		
			NsiEco5		NsiEco6		NsiEco7A		98lys7B		NsiEco8		
			SEQ ID NO:540		SEQ ID NO:541		SEQ ID NO:543		SEQ ID NO:459		SEQ ID NO:545		
98 his	arg	CGT	NsiEco1		NsiEco2		NsiEco3A		98arg3B		NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534		SEQ ID NO:535		SEQ ID NO:537		SEQ ID NO:456		SEQ ID NO:539		
			NsiEco5		NsiEco6		NsiEco7A		98arg7B		NsiEco8		
			SEQ ID NO:540		SEQ ID NO:541		SEQ ID NO:543		SEQ ID NO:457		SEQ ID NO:545		
98 his	tyr	TAC	NsiEco1		NsiEco2		NsiEco3A		98tyr3B		NsiEco4	pMON13358	NsiI,EcoRI
			SEQ ID NO:534		SEQ ID NO:535		SEQ ID NO:537		SEQ ID NO:460		SEQ ID NO:539		
			NsiEco5		NsiEco6		NsiEco7A		98tyr7B		NsiEco8		
			SEQ ID NO:540		SEQ ID NO:541		SEQ ID NO:543		SEQ ID NO:461		SEQ ID NO:545		
101 asp	glu	GAA	NsiEco1		NsiEco2		NsiEco3		101glu4			pMON13358	NsiI,EcoRI
			SEQ ID NO:534		SEQ ID NO:535		SEQ ID NO:536		SEQ ID NO:466				
			NsiEco5		NsiEco6		NsiEco7		101glu8				
			SEQ ID NO:540		SEQ ID NO:541		SEQ ID NO:542		SEQ ID NO:467				
101 asp	asn	AAC	NsiEco1		NsiEco2		NsiEco3		101asn4			pMON13358	NsiI,EcoRI
			SEQ ID NO:534		SEQ ID NO:535		SEQ ID NO:536		SEQ ID NO:464				
			NsiEco5		NsiEco6		NsiEco7		101asn8				
			SEQ ID NO:540		SEQ ID NO:541		SEQ ID NO:542		SEQ ID NO:465				
101 asp	ser	TCC	NsiEco1		NsiEco2		NsiEco3		101ser4			pMON13358	NsiI,EcoRI
			SEQ ID NO:534		SEQ ID NO:535		SEQ ID NO:536		SEQ ID NO:476				
			NsiEco5		NsiEco6		NsiEco7		101ser8				
			SEQ ID NO:540		SEQ ID NO:541		SEQ ID NO:542		SEQ ID NO:477				
101 asp	ala	GCT	NsiEco1		NsiEco2		NsiEco3		101ala4			pMON13358	NsiI,EcoRI
			SEQ ID NO:534		SEQ ID NO:535		SEQ ID NO:536		SEQ ID NO:462				
			NsiEco5		NsiEco6		NsiEco7		101ala8				
			SEQ ID NO:540		SEQ ID NO:541		SEQ ID NO:542		SEQ ID NO:463				
101 asp	gly	GGT	NsiEco1		NsiEco2		NsiEco3		101gly4			pMON13358	NsiI,EcoRI
			SEQ ID NO:534		SEQ ID NO:535		SEQ ID NO:536		SEQ ID NO:468				
			NsiEco5		NsiEco6		NsiEco7		101gly8				
			SEQ ID NO:540		SEQ ID NO:541		SEQ ID NO:542		SEQ ID NO:469				

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
101 asp	ile	ATC	NsiEco1	NsiEco2	NsiEco3	101ile4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:536	SEQ ID NO:470			
			NsiEco5	NsiEco6	NsiEco7	101ile8			
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:542	SEQ ID NO:471			
101 asp	leu	CTG	NsiEco1	NsiEco2	NsiEco3	101leu4		pMON13358	NsiI,EcoRI
			SEQ ID NO:534	SEQ ID NO:535	SEQ ID NO:536	SEQ ID NO:472			
			NsiEco5	NsiEco6	NsiEco7	101leu8			
			SEQ ID NO:540	SEQ ID NO:541	SEQ ID NO:542	SEQ ID NO:473			
108 arg	gln	CAG	108gln1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:480	SEQ ID NO:547					
			108gln3	EcoHin4					
			SEQ ID NO:481	SEQ ID NO:549					
108 arg	his	CAC	108his1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:482	SEQ ID NO:547					
			108his3	EcoHin4					
			SEQ ID NO:483	SEQ ID NO:549					
108 arg	ser	TCC	108ser1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:484	SEQ ID NO:547					
			108ser3	EcoHin4					
			SEQ ID NO:485	SEQ ID NO:549					
108 arg	ala	GCT	108ala1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:478	SEQ ID NO:547					
			108ala3	EcoHin4					
			SEQ ID NO:479	SEQ ID NO:549					
110 lys	arg	CGT	110arg1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO486	SEQ ID NO:547					
			110arg3	EcoHin4					
			SEQ ID NO:487	SEQ ID NO:549					
110 lys	his	CAC	110his1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:490	SEQ ID NO:547					
			110his3	EcoHin4					
			SEQ ID NO:491	SEQ ID NO:549					

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
110 lys	glu	GAA	110glu1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:488	SEQ ID NO:547					
			110glu3	EcoHin4					
			SEQ ID NO:489	SEQ ID NO:549					
110 lys	ser	TCC	110ser1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:494	SEQ ID NO:547					
			110ser3	EcoHin4					
			SEQ ID NO:495	SEQ ID NO:549					
110 lys	ala	GCT	110ala1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:492	SEQ ID NO:547					
			110ala3	EcoHin4					
			SEQ ID NO:493	SEQ ID NO:549					
113 phe	asp	GAC	113asp1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:498	SEQ ID NO:547					
			113asp3	EcoHin4					
			SEQ ID NO:497	SEQ ID NO:549					
113 phe	lys	AAA	113lys1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:502	SEQ ID NO:547					
			113lys3	EcoHin4					
			SEQ ID NO:503	SEQ ID NO:549					
113 phe	leu	CTG	113leu1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:500	SEQ ID NO:547					
			113leu3	EcoHin4					
			SEQ ID NO:501	SEQ ID NO:549					
113 phe	ile	ATC	113ile1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:498	SEQ ID NO:547					
			113ile3	EcoHin4					
			SEQ ID NO:499	SEQ ID NO:549					
113 phe	val	GTT	113val1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:504	SEQ ID NO:547					
			113val3	EcoHin4					
			SEQ ID NO:505	SEQ ID NO:549					

TABLE 8

position/native a.a.	substitution	codon	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	parental plasmid	restriction digest
116 lys	asn	AAC	116asn1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:510	SEQ ID NO:547					
			116asn3	EcoHin4					
			SEQ ID NO:511	SEQ ID NO:549					
116 lys	arg	CGT	116arg1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:508	SEQ ID NO:547					
			116arg3	EcoHin4					
			SEQ ID NO:509	SEQ ID NO:549					
116 lys	his	CAC	116his1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:514	SEQ ID NO:547					
			116his3	EcoHin4					
			SEQ ID NO:515	SEQ ID NO:549					
116 lys	ala	GCT	116ala1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:506	SEQ ID NO:547					
			116ala3	EcoHin4					
			SEQQ ID NO:507	SEQ ID NO:549					
116 lys	tyr	TAC	116tyr1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:520	SEQ ID NO:547					
			116tyr3	EcoHin4					
			SEQ ID NO:521	SEQ ID NO:549					
116 lys	phe	TTC	116phe1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:518	SEQ ID NO:547					
			116phe3	EcoHin4					
			SEQ ID NO:519	SEQ ID NO:549					
116 lys	gln	CAG	116gln1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:512	SEQ ID NO:547					
			116gln3	EcoHin4					
			SEQ ID NO:513	SEQ ID NO:549					
116 lys	met	ATG	116met1	EcoHin2				pMON13359	EcoRI,HinDIII
			SEQ ID NO:516	SEQ ID NO:547					
			116met3	EcoHin4					
			SEQ ID NO:517	SEQ ID NO:549					

It will be apparent to those skilled in the art that other codons besides those shown in Table 8 can also code for the substituted amino acids in the hIL-3 muteins. The present invention includes the DNAs encoding the mutant hIL-3 polypeptides of the invention including the various codons which can code for the parental and substituted amino acids of the hIL-3 muteins of the invention due to the degeneracy of the genetic code.

hIL-3 (15-125) variant genes encoding the variants listed in Table 8 can also be expressed from intracellular expression vectors to produce large quantities of the variant protein which can be purified and assayed for biological activity. The hIL-3 variant genes, from Table 8, can be excised from the secretion expression vector, as a 345 base pair NcoI/HindIII fragment and ligated into an appropriate intrecellular expression vector, such as pMON2341 digested with NcoI and HindIII.

Table 9 shows the biological activity of (15-125)hIL-3 muteins of the present invention which have one amino acid substitutions in the (15-125)hIL-3 polypeptide and which were constructed as described in Example 66. The mutants in Table 9 were secreted into the periplasmic space in E.coli. The periplasmic content was released by osmotic shock and the material in the crude osmotic shock fraction was screened for growth promoting activity. Biological activity is the growth promoting activity of AML cells relative to (15-125) hIL-3 (pMON6458 or pMMON5988). The relative biological activity of IL-3 mutants is calculated by dividing the EC_{50} (1-133) hIL-3 by the EC_{50} of the mutant. The numbers in parentheses indicate the number of repeat assays. When a variant was assayed more than once the standard deviation is indicated. An "*" indicates that the hIL3 variant protein level was less than 1.0 ug/ml and was not screened for growth promoting activity.

TABLE 9

PARENTAL			(15-125) hIL-3 MUTANT			
aa position	AA	codon	AA	SEQ ID NO:	codon	BIOL ACTIVITY
21/7	ASP	GAT	ASN	19	AAC	0.01
21/7	ASP	GAT	GLN	19	CAA	0.07
21/7	ASP	GAT	GLU	19	GAA	0.5
21/7	ASP	GAT	SER	19	AGC	0.1
21/7	ASP	GAT	THR	19	ACC	0.1
22/8	GLU	GAA	ASN	19	AAC	*
22/8	GLU	GAA	ASP	19	GAC	*
22/8	GLU	GAA	GLN	19	CAG	< 0.01
22/8	GLU	GAA	LEU	19	CTG	*
22/8	GLU	GAA	VAL	19	GTT	*
34/20	LEU	TTG	ALA	19	GCT	2.2
34/20	LEU	TTG	ARG	19	CGT	2.2
34/20	LEU	TTG	GLN	19	CAG	1.1
34/20	LEU	TTG	GLU	19	GAA	1.5
34/20	LEU	TTG	ILE	19	ATC	1.3
34/20	LEU	TTG	PHE	19	TTC	1.8
34/20	LEU	TTG	THR	19	ACC	1.1
42/28	GLY	GGG	ASN	19	AAC	1.3 (3) 0.28
42/28	GLY	GGG	ILE	19	ATC	10
42/28	GLY	GGG	LEU	19	CTG	10.1 (3) 7.57
42/28	GLY	GGG	MET	19	ATG	2.2 (3) 1.14
42/28	GLY	GGG	TYR	19	TAC	11 (2) 8.9
42/28	GLY	GGG	VAL	19	GTT	0.33
43/29	GLU	GAA	ARG	19	CGT	*
43/29	GLU	GAA	GLN	19	CAG	<0.004
43/29	GLU	GAA	GLY	19	GGT	*
43/29	GLU	GAA	THR	19	ACC	0.005
44/30	ASP	GAC	ALA	19	GCT	*
44/30	ASP	GAC	ASN	19	AAC	*
44/30	ASP	GAC	GLN	19	CAG	*
44/30	ASP	GAC	GLU	19	GAA	0.66

TABLE 9

PARENTAL			(15-125) hIL-3 MUTANT			
aa position	AA	codon	AA	SEQ ID NO:	codon	BIOL ACTIVITY
45/31	GLN	CAA	ALA	19	GCT	1
45/31	GLN	CAA	ASN	19	AAC	15.8
45/31	GLN	CAA	GLU	19	GAA	2.3
45/31	GLN	CAA	ILE	19	ATC	4.9
45/31	GLN	CAA	SER	19	TCC	0.7
46/32	ASP	GAC	ALA	19	GCT	6.3
46/32	ASP	GAC	ASN	19	AAC	0.66, 1.1
46/32	ASP	GAC	GLN	19	CAG	6.3
46/32	ASP	GAC	GLU	19	GAA	1.97 (3) 2.14
46/32	ASP	GAC	HIS	19	CAC	3.2, 1.4
46/32	ASP	GAC	ILE	19	ATC	0.5
46/32	ASP	GAC	LYS	19	AAA	0.5
46/32	ASP	GAC	TYR	19	TAC	0.66
46/32	ASP	GAC	VAL	19	GTT	6.3
48/34	LEU	CTG	GLU	19	GAA	*
48/34	LEU	CTG	HIS	19		*
48/34	LEU	CTG	LYS	19	AAA	*
48/34	LEU	CTG	THR	19	ACC	*
48/34	LEU	CTG	VAL	19	CAC	*
50/36	GLU	GAA	ALA	19	GCT	0.5
50/36	GLU	GAA	ASN	19	AAC	1.7
50/36	GLU	GAA	HIS	19	CAC	*
50/36	GLU	GAA	LYS	19	AAA	*
50/36	GLU	GAA	SER	19	TCC	1.3
50/36	GLU	GAA	VAL	19	GTT	*
54/40	ARG	CGA	ALA	19	GCT	0.9
54/40	ARG	CGA	ASN	19	AAC	*
54/40	ARG	CGA	HIS	19	CAC	0.01
54/40	ARG	CGA	LYS	19	AAA	0.2

TABLE 9

PARENTAL			(15-125) hIL-3 MUTANT			
aa position	AA	codon	AA	SEQ ID NO:	codon	BIOL ACTIVITY
56/42	PRO	CAA	ALA	19	GCT	1.8
56/42	PRO	CAA	ASN	19		0.6
56/42	PRO	CAA	ARG	19	CGT	1.2
56/42	PRO	CAA	GLU	19	GAA	0.9
56/42	PRO	CAA	HIS	19	CAC	0.4
56/42	PRO	CAA	LEU	19	CTG	1.2
56/42	PRO	CAA	PHE	19	TTC	
56/42	PRO	CAA	THR	19	ACC	0.6
56/42	PRO	CAA	VAL	19	GTT	1.1
82/68	LEU	CTG	ALA	19	GCT	0.5
82/68	LEU	CTG	ASN	19	AAC	2.9
82/68	LEU	CTG	GLU	19	GAA	4.57 (3) 5.0
82/68	LEU	CTG	HIS	19	CAC	2.2
82/68	LEU	CTG	ILE	19	ATC	0.8
82/68	LEU	CTG	MET	19	ATG	1.1
82/68	LEU	CTG	PHE	19	TTC	3.2
82/68	LEU	CTG	SER	19	TCC	2.2
82/68	LEU	CTG	THR	19	ACC	1.6
82/68	LEU	CTG	TYR	19	TAC	2.7
94/80	ARG	CGA	GLN	19	CAG	0.03
94/80	ARG	CGA	HIS	19	CAC	0.01
94/80	ARG	CGA	LYS	19	AAA	*
95/81	HIS	CAT	ASN	19	AAC	2.7 (2) 2.3
95/81	HIS	CAT	ILE	19	ATC	0.33
95/81	HIS	CAT	LYS	19	AAA	0.9
95/81	HIS	CAT	MET	19	ATG	1
95/81	HIS	CAT	PHE	19	TTC	0.66
95/81	HIS	CAT	SER	19	TCC	4
95/81	HIS	CAT	TRP	19	TGG	*

TABLE 9

PARENTAL			(15-125) hIL-3 MUTANT			
aa position	AA	codon	AA	SEQ ID NO:	codon	BIOL ACTIVITY
98/84	HIS	CAT	ARG	19	CGT	3.2
98/84	HIS	CAT	GLN	19	CAA	2.2
98/84	HIS	CAT	GLU	19	GAA	1.55 (2) 0.15
98/84	HIS	CAT	LYS	19	AAA	4
98/84	HIS	CAT	MET	19	ATG	2.2
98/84	HIS	CAT	PHE	19	TTC	1
98/84	HIS	CAT	SER	19	TCC	4
98/84	HIS	CAT	THR	19		2.2
98/84	HIS	CAT	VAL	19	GTA	2.4 (2) 0.8
101/87	ASP	GAC	ASN	19	AAC	7
101/87	ASP	GAC	GLU	19	GAA	*
101/87	ASP	GAC	ILE	19	ATC	3.2
101/87	ASP	GAC	LEU	19	CTG	3.2
108/94	ARG	CGG	ALA	19	GCT	4
108/94	ARG	CGG	GLN	19	CAG	0.4
108/94	ARG	CGG	HIS	19	CAC	*
108/94	ARG	CGG	SER	19	TCC	3.7
110/96	LYS	AAA	GLU	19	GAA	*
110/96	LYS	AAA	HIS	19	CAC	*
110/96	LYS	AAA	ILE	19	ATC	*
113/99	PHE	TTC	ASP	19	GAC	*
113/99	PHE	TTC	ILE	19	ATC	*
113/99	PHE	TTC	LEU	19	CTG	*
113/99	PHE	TTC	LYS	19	AAA	*
116/102	LYS	AAA	ALA	19	GCT	5
116/102	LYS	AAA	ARG	19	CGT	0.03
116/102	LYS	AAA	ASN	19	AAC	0.22
116/102	LYS	AAA	GLN	19	CAG	0.33
116/102	LYS	AAA	HIS	19	CAC	3.2
116/102	LYS	AAA	MET	19	ATG	0.9
116/102	LYS	AAA	PHE	19	TTC	2.5
116/102	LYS	AAA	TYR	19	TAC	5.4 (2) 0.3

WHAT IS CLAIMED IS:

1. A human interleukin-3 mutant polypeptide of the Formula I:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
80 85 90

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
95 100 105

Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:15]
125 130

wherein;

Xaa at position 17 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;

Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;

Xaa at position 19 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;

Xaa at position 20 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;

Xaa at position 21 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn, Thr, Ser or Val;

Xaa at position 22 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln, Leu, Val or Gly;

Xaa at position 23 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe, Leu, Ser, or Arg;

Xaa at position 24 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;

Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;

Xaa at position 26 is His, Thr, Phe, Gly, Arg, Ala, or Trp;

Xaa at position 27 is Leu, Gly, Arg, Thr, Ser, or Ala;

Xaa at position 28 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;

Xaa at position 29 is Gln, Asn, Leu, Pro, Arg, or Val;

Xaa at position 30 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;

Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;

Xaa at position 32 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 33 is Pro, Leu, Gln, Ala, Thr, or Glu;

Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr, Arg, Ala, Phe, Ile or Met;

Xaa at position 35 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;

Xaa at position 36 is Asp, Leu, or Val;

Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;

Xaa at position 38 is Asn, or Ala;

Xaa at position 40 is Leu, Trp, or Arg;

Xaa at position 41 is Asn, Cys, Arg, Leu, His, Met, or Pro;

Xaa at position 42 is Gly, Asp, Ser, Cys, Asn, Lys, Thr, Leu, Val, Glu, Phe, Tyr, Ile, Met or Ala;

Xaa at position 43 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, Gln, Arg, Thr, Gly or Ser;

Xaa at position 44 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, Glu, Asn, Gln, Ala or Pro;

Xaa at position 45 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, Trp, Asp, Asn, Arg, Ser, Ala, Ile, Glu or His;

Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln, Lys, His, Ala, Tyr, Ile, Val or Gly;

Xaa at position 47 is Ile, Gly, Val, Ser, Arg, Pro, or His;

Xaa at position 48 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu, Lys, Thr, Ala, Met, Val or Asn;

Xaa at position 49 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;
Xaa at position 50 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser, Ala, Ile, Val, His, Phe, Met or Gln;
Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 52 is Asn, His, Arg, Leu, Gly, Ser, or Thr;
Xaa at position 53 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser, or Met;
Xaa at position 54 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn, Lys, His, Ala or Leu;
Xaa at position 55 is Arg, Thr, Val, Ser, Leu, or Gly;
Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His, Thr, Ala, Tyr, Phe, Leu, Val or Lys;
Xaa at position 57 is Asn or Gly;
Xaa at position 58 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
Xaa at position 59 is Glu Tyr, His, Leu, Pro, or Arg;
Xaa at position 60 is Ala, Ser, Pro, Tyr, Asn, or Thr;
Xaa at position 61 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
Xaa at position 62 is Asn His, Val, Arg, Pro, Thr, Asp, or Ile;
Xaa at position 63 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or Val;
Xaa at position 64 is Ala, Asn, Pro, Ser, or Lys;
Xaa at position 65 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 67 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or His;
Xaa at position 68 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;
Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or Leu;
Xaa at position 70 is Asn, Leu, Val, Trp, Pro, or Ala;
Xaa at position 71 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln, Trp, or Asn;
Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;
Xaa at position 74 is Ile, Met, Thr, Pro, Arg, Gly, Ala;
Xaa at position 75 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser, Gln, or Leu;
Xaa at position 76 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or Asp;
Xaa at position 77 is Ile, Ser, Arg, Thr, or Leu;

Xaa at position 78 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;
Xaa at position 79 is Lys, Thr, Asn, Met, Arg, Ile, Gly, or Asp;
Xaa at position 80 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 81 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;
Xaa at position 82 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn, His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;
Xaa at position 83 is Pro, Ala, Thr, Trp, Arg, or Met;
Xaa at position 84 is Cys, Glu, Gly, Arg, Met, or Val;
Xaa at position 85 is Leu, Asn, Val, or Gln;
Xaa at position 86 is Pro, Cys, Arg, Ala, or Lys;
Xaa at position 87 is Leu, Ser, Trp, or Gly;
Xaa at position 88 is Ala, Lys, Arg, Val, or Trp;
Xaa at position 89 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn, or Ser;
Xaa at position 90 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or Met;
Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;
Xaa at position 92 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile or Leu;
Xaa at position 93 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;
Xaa at position 94 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys, His, Ala, or Pro;
Xaa at position 95 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr, Asn, Lys, Ser, Ala, Trp, Phe, Ile, or Tyr;
Xaa at position 96 is Pro, Lys, Tyr, Gly, Ile, or Thr;
Xaa at position 97 is Ile, Val, Lys, Ala, or Asn;
Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr, Glu, Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;
Xaa at position 99 is Ile, Leu, Arg, Asp, Val, Pro, Gln, Gly, Ser, Phe, or His;
Xaa at position 100 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln, or Pro;
Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val, Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu, or Gln;
Xaa at position 102 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
Xaa at position 103 is Asp, or Ser;
Xaa at position 104 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu, Gln, Lys, Ala, Phe, or Gly;

Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
Xaa at position 106 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;
Xaa at position 108 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His, Ser, Ala or Pro;
Xaa at position 109 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;
Xaa at position 110 is Lys, Ala, Asn, Thr, Leu, Arg, Gln, His, Glu, Ser, Ala, or Trp;
Xaa at position 111 is Leu, Ile, Arg, Asp, or Met;
Xaa at position 112 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;
Xaa at position 113 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp, Lys, Leu, Ile, Val or Asn;
Xaa at position 114 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;
Xaa at position 115 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr, Trp, or Met;
Xaa at position 116 is Lys, Leu, Pro, Thr, Met, Asp, Val, Glu, Arg, Trp, Ser, Asn, His, Ala, Tyr, Phe, Gln, or Ile;
Xaa at position 117 is Thr, Ser, Asn, Ile, Trp, Lys, or Pro;
Xaa at position 118 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;
Xaa at position 119 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;
Xaa at position 120 is Asn, Ala, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or Gly;
Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3 with the proviso that when Xaa at position 22 is Leu, and/or Xaa at position 34 is Gly or Glu, and/or Xaa at position 44 is Ala, and/or Xaa at position 46 is Lys or Ala, and/or Xaa at position 50 is Lys, and/or Xaa at position 59 is Pro or Arg, and/or Xaa at position 63 is Lys, and/or Xaa at position 75 is Gly or Arg, and/or Xaa at position 94 is Pro,

and/or Xaa at position 98 is Arg, and/or Xaa at position 106 is Lys, and/or Xaa at position 110 is Ala or Glu, and/or Xaa at position 111 is Met, then there must be at least one additional substitution besides the ones indicated.

2. A human interleukin-3 mutant polypeptide of the Formula II:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Xaa Xaa Xaa Xaa Xaa Glu Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa Xaa Glu Xaa Xaa
35 40 45

Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa Xaa
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75

Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Cys Xaa Pro Xaa Xaa Xaa Xaa
80 85 90

Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa Xaa
95 100 105

Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Xaa Xaa
110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:16]
125 130

wherein

Xaa at position 17 is Ser, Gly, Asp, Met, or Gln;

Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
Xaa at position 19 is Met, Phe, Ile, Arg, or Ala;
Xaa at position 20 is Ile or Pro;
Xaa at position 21 is Asp or Glu;
Xaa at position 23 is Ile, Val, Ala, Leu, or Gly;
Xaa at position 24 is Ile, Val, Phe, or Leu;
Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
Xaa at position 26 is His, Phe, Gly, Arg, or Ala;
Xaa at position 28 is Lys, Leu, Gln, Gly, Pro, or Val;
Xaa at position 29 is Gln, Asn, Leu, Arg, or Val;
Xaa at position 30 is Pro, His, Thr, Gly, or Gln;
Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;
Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 33 is Pro, Leu, Gln, Ala, or Glu;
Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln, Glu, Ile, Phe, Thr or Met;
Xaa at position 35 is Leu, Ala, Asn, Pro, Gln, or Val;
Xaa at position 36 is Asp or Leu;
Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;
Xaa at position 38 is Asn or Ala;
Xaa at position 41 is Asn, Cys, Arg, His, Met, or Pro;
Xaa at position 42 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu, Met, Tyr, Val or Arg;
Xaa at position 44 is Asp or Glu;
Xaa at position 45 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn, Glu, Ser, or Trp;
Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln, Glu, His, Ile, Lys, Tyr, Val or Gly;
Xaa at position 47 is Ile, Val, or His;
Xaa at position 49 is Met, Asn, or Asp;
Xaa at position 50 is Glu, Thr, Ala, Asn, Ser or Asp;
Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 52 is Asn or Gly;
Xaa at position 53 is Leu, Met, or Phe;
Xaa at position 54 is Arg, Ala, or Ser;
Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;
Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn, Glu, His, Leu, Thr, Val or Lys;

Xaa at position 59 is Glu, Tyr, His, Leu, or Arg;
Xaa at position 60 is Ala, Ser, Asn, or Thr;
Xaa at position 61 is Phe or Ser;
Xaa at position 62 is Asn, Val, Pro, Thr, or Ile;
Xaa at position 63 is Arg, Tyr, Lys, Ser, His, or Val;
Xaa at position 64 is Ala or Asn;
Xaa at position 65 is Val, Thr, Leu, or Ser;
Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 67 is Ser, Phe, Val, Gly, Asn, Ile, or His;
Xaa at position 68 is Leu, Val, Ile, Phe, or His;
Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
Xaa at position 70 is Asn or Pro;
Xaa at position 71 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;
Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or Pro;
Xaa at position 74 is Ile or Met;
Xaa at position 75 is Glu, Gly, Asp, Ser, or Gln;
Xaa at position 76 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or Asp;
Xaa at position 77 is Ile, Ser, or Leu;
Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or Asp;
Xaa at position 80 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 81 is Leu, or Val;
Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 83 is Pro, Ala, Thr, Trp, or Met;
Xaa at position 85 is Leu or Val;
Xaa at position 87 is Leu or Ser;
Xaa at position 88 is Ala, Arg, or Trp;
Xaa at position 89 is Thr, Asp, Glu, His, Asn, or Ser;
Xaa at position 90 is Ala, Asp, or Met;
Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;
Xaa at position 92 is Pro or Ser;
Xaa at position 93 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
Xaa at position 95 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile, Phe, Ser or Thr;

Xaa at position 96 is Pro or Tyr;
Xaa at position 97 is Ile, Val, or Ala;
Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr, Leu, Arg, Gln, Glu, lys, Met, Ser, Tyr, Val or Pro;
Xaa at position 99 is Ile, Leu, Val, or Phe;
Xaa at position 100 is Lys, Leu, His, Arg, Ile, Gln, Pro, or Ser;
Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val, Asn, Ile, Leu or Tyr;
Xaa at position 102 is Gly, Glu, Lys, or Ser;
Xaa at position 104 is Trp, Val, Tyr, Met, or Leu;
Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
Xaa at position 106 is Glu, Ser, Ala, or Gly;
Xaa at position 108 is Arg, Ala, Gln, Ser or Lys;
Xaa at position 109 is Arg, Thr, Glu, Leu, Ser, or Gly;
Xaa at position 112 is Thr, Val, Gln, Glu, His, or Ser;
Xaa at position 114 is Tyr or Trp;
Xaa at position 115 is Leu or Ala;
Xaa at position 116 is Lys, Thr, Met, Val, Trp, Ser, Leu, Ala, Asn, Gln, His, Met, Phe, Tyr or Ile;
Xaa at position 117 is Thr, Ser, or Asn;
Xaa at position 119 is Glu, Ser, Pro, Leu, Thr, or Tyr;
Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or Gly;
Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3 with the proviso that when Xaa at position 34 is Gly or Xaa or position 46 is Lys or Ala or/and Xaa at position 59 is Arg and/or Xaa at position 63 is Lys

and/or Xaa at position 75 is Gly and/or Xaa at position 98 is Arg then there must be at least one additional substitution besides the ones indicated.

3. A human interleukin-3 mutant polypeptide according to claim 2 of the Formula III:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Xaa Xaa Xaa Ile Xaa Glu Xaa Xaa Xaa Xaa Leu Lys Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Asp Xaa Xaa Asn Leu Asn Xaa Glu Xaa Xaa
35 40 45

Xaa Ile Leu Met Xaa Xaa Asn Leu Xaa Xaa Xaa Asn Leu Glu Xaa
50 55 60

Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Ile Glu
65 70 75

Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Cys Xaa Pro Xaa Xaa Thr Ala
80 85 90

Xaa Pro Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Asp Xaa Xaa
95 100 105

Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Glu Xaa
110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:17]
125 130

wherein
Xaa at position 17 is Ser, Gly, Asp, Met, or Gln;
Xaa at position 18 is Asn, His, or Ile;
Xaa at position 19 is Met or Ile;

Xaa at position 21 is Asp or Glu;
Xaa at position 23 is Ile, Ala, Leu, or Gly;
Xaa at position 24 is Ile, Val, or Leu;
Xaa at position 25 is Thr, His, Gln, or Ala;
Xaa at position 26 is His or Ala;
Xaa at position 29 is Gln, Asn, or Val;
Xaa at position 30 is Pro, Gly, or Gln;
Xaa at position 31 is Pro, Asp, Gly, or Gln;
Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 33 is Pro or Glu;
Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln, Glu, Ile, Phe, Thr or Met;
Xaa at position 35 is Leu, Ala, Asn, Pro, Gln, or Val;
Xaa at position 37 is Phe, Ser, Pro, or Trp;
Xaa at position 38 is Asn or Ala;
Xaa at position 42 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu, Met, Tyr or Arg;
Xaa at position 44 is Asp or Glu;
Xaa at position 45 is Gln, Val, Met, Leu, Thr, Ala, Asn, Glu, Ser or Lys;
Xaa at position 46 is Asp, Phe, Ser, Thr, Ala, Asn Gln, Glu, His, Ile, Lys, Tyr, Val or Cys;
Xaa at position 50 is Glu, Ala, Asn, Ser or Asp;
Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 54 is Arg or Ala;
Xaa at position 54 is Arg or Ala;
Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;
Xaa at position 56 is Pro, Gly, Ser, Gln, Ala, Arg, Asn, Glu, Leu, Thr, Val or Lys;
Xaa at position 60 is Ala or Ser;
Xaa at position 62 is Asn, Pro, Thr, or Ile;
Xaa at position 63 is Arg or Lys;
Xaa at position 64 is Ala or Asn;
Xaa at position 65 is Val or Thr;
Xaa at position 66 is Lys or Arg;
Xaa at position 67 is Ser, Phe, or His;
Xaa at position 68 is Leu, Ile, Phe, or His;
Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;

Xaa at position 71 is Ala, Pro, or Arg;
Xaa at position 72 is Ser, Glu, Arg, or Asp;
Xaa at position 73 is Ala or Leu;
Xaa at position 76 is Ser, Val, Ala, Asn, Glu, Pro, or Gly;
Xaa at position 77 is Ile or Leu;
Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or Asp;
Xaa at position 80 is Asn, Gly, Glu, or Arg;
Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His, Ile, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 83 is Pro or Thr;
Xaa at position 85 is Leu or Val;
Xaa at position 87 is Leu or Ser;
Xaa at position 88 is Ala or Trp;
Xaa at position 91 is Ala or Pro;
Xaa at position 93 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
Xaa at position 95 is His, Pro, Arg, Val, Leu, Gly, Asn, Phe, Ser or Thr;
Xaa at position 96 is Pro or Tyr;
Xaa at position 97 is Ile or Val;
Xaa at position 98 is His, Ile, Asn, Leu, Ala, Thr, Leu, Arg, Gln, Leu, Lys, Met, Ser, Tyr, Val or Pro;
Xaa at position 99 is Ile, Leu, or Val;
Xaa at position 100 is Lys, Arg, Ile, Gln, Pro, or Ser;
Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Pro, Asn, Ile, Leu or Tyr;
Xaa at position 104 is Trp or Leu;
Xaa at position 105 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
Xaa at position 106 is Glu or Gly;
Xaa at position 108 is Arg, Ala, or Ser;
Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;
Xaa at position 112 is Thr, Val, or Gln;
Xaa at position 114 is Tyr or Trp;
Xaa at position 115 is Leu or Ala;
Xaa at position 116 is Lys, Thr, Val, Trp, Ser, Ala, His, Met, Phe, Tyr or Ile;
Xaa at position 117 is Thr or Ser;

Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;
Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133)human interleukin-3 with the proviso that when Xaa at position 34 is Gly and/or Xaa at position 46 is Lys or Ala, and/or Xaa at position 63 is Lys, and/or Xaa at position 98 is Arg, then two or three of the amino acid designated by Xaa are different from the corresponding amino acids of the native (1-133) human interleukin-3.

4. A human interleukin-3 mutant polypeptide according to Claim 3 of the Formula IV:

```
Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
 1               5                  10                  15

Cys Xaa Xaa Met Ile Asp Glu Xaa Ile Xaa Xaa Leu Lys Xaa Xaa
                20                  25                  30

Pro Xaa Pro Xaa Xaa Asp Phe Xaa Asn Leu Asn Xaa Glu Asp Xaa
                35                  40                  45

Xaa Ile Leu Met Xaa Xaa Asn Leu Arg Xaa Xaa Asn Leu Glu Ala
                50                  55                  60

Phe Xaa Arg Xaa Xaa Lys Xaa Xaa Xaa Asn Ala Ser Ala Ile Glu
                65                  70                  75

Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Cys Leu Pro Xaa Xaa Thr Ala
                80                  85                  90
```

Xaa Pro Xaa Arg Xaa Pro Ile Xaa Xaa Xaa Xaa Gly Asp Trp Xaa
95 100 105

Glu Phe Xaa Xaa Lys Leu Xaa Phe Tyr Leu Xaa Xaa Leu Glu Xaa
110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:18]
125 130

wherein
Xaa at position 17 is Ser, Gly, Asp, or Gln;
Xaa at position 18 is Asn, His, or Ile;
Xaa at position 23 is Ile, Ala, Leu, or Gly;
Xaa at position 25 is Thr, His, or Gln;
Xaa at position 26 is His or Ala;
Xaa at position 29 is Gln or Asn;
Xaa at position 30 is Pro or Gly;
Xaa at position 32 is Leu, Arg, Asn, or Ala;
Xaa at position 34 is Leu, Val, Ser, Ala, Arg, Gln, Glu, Ile, Phe, Thr, or Met;
Xaa at position 35 is Leu, Ala, Asn, or Pro;
Xaa at position 38 is Asn or Ala;
Xaa at position 42 is Gly, Asp, Ser, Ala, Asn, Ile, Leu, Met, Tyr or Arg;
Xaa at position 45 is Gln, Val, Met, Leu, Ala, Asn, Glu, or Lys;
Xaa at position 46 is Asp, Phe, Ser, Gln, Glu, His, Val or Thr;
Xaa at position 50 is Glu Asn, Ser or Asp;
Xaa at position 51 is Asn, Arg, Pro, Thr, or His;
Xaa at position 55 is Arg, Leu, or Gly;
Xaa at position 56 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;
Xaa at position 62 is Asn, Pro, or Thr;
Xaa at position 64 is Ala or Asn;
Xaa at position 65 is Val or Thr;
Xaa at position 67 is Ser or Phe;
Xaa at position 68 is Leu or Phe;
Xaa at position 69 is Gln, Ala, Glu, or Arg;
Xaa at position 76 is Ser, Val, Asn, Pro, or Gly;

Xaa at position 77 is Ile or Leu;
Xaa at position 79 is Lys, Gly, Asn, Met, Arg, Ile, or Gly;
Xaa at position 80 is Asn, Gly, Glu, or Arg;
Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 87 is Leu or Ser;
Xaa at position 88 is Ala or Trp;
Xaa at position 91 is Ala or Pro;
Xaa at position 93 is Thr, Asp, or Ala;
Xaa at position 95 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;
Xaa at position 98 is His, Ile, Asn, Ala, Thr, Gln, Glu, Lys, Met, Ser, Tyr, Val or Leu;
Xaa at position 99 is Ile or Leu;
Xaa at position 100 is Lys or Arg;
Xaa at position 101 is Asp, Pro, Met, Lys, Thr, His, Pro, Asn, Ile, Leu or Tyr;
Xaa at position 105 is Asn, Pro, Ser, Ile or Asp;
Xaa at position 108 is Arg, Ala, or Ser;
Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;
Xaa at position 112 is Thr or Gln;
Xaa at position 116 is Lys, Val, Trp, Ala, His, Phe, Tyr or Ile;
Xaa at position 117 is Thr or Ser;
Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Pro, or Asp;
Xaa at position 122 is Gln, Met, Trp, Phe, Pro, His, Ile, or Tyr;
Xaa at position 123 is Ala, Met, Glu, Ser, or Leu;

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133)human interleukin-3.

5. A human interleukin-3 mutant polypeptide according to claim 4 wherein said polypeptide has Ala at position 64.

6. The human interleukin-3 mutant polypeptide acording to

Claim 1 wherein:

Xaa at position 17 is Ser, Lys, Asp, Met, Gln, or Arg;
Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
Xaa at position 19 is Met, Arg, Gly, Ala, or Cys;
Xaa at position 20 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;
Xaa at position 21 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Thr, Cer or Val;
Xaa at position 22 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Val or Gly;
Xaa at position 23 is Ile, Ala, Gly, Trp, Lys, Leu, Ser, or Arg;
Xaa at position 24 is Ile, Gly, Arg, or Ser;
Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
Xaa at position 26 is His, Thr, Phe, Gly, Ala, or Trp;
Xaa at position 27 is Leu, Gly, Arg, Thr, Ser, or Ala;
Xaa at position 28 is Lys, Leu, Gln, Gly, Pro, Val or Trp;
Xaa at position 29 is Gln, Asn, Loh, Pro, Arg, or Val;
Xaa at position 30 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;
Xaa at position 31 is Pro, Asp, Gly, Arg, Leu, or Gln;
Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 33 is Pro, Leu, Gln, Thr, or Glu;
Xaa at position 34 is Leu, Ser, Gln, Thr, Arg, Ala, or Lys;
Xaa at position 35 is Leu, Ala, Gly, Asn, Pro, or Gln;
Xaa at position 36 is Asp, Leu, or Val;
Xaa at position 37 is Phe, Ser, or Pro;
Xaa at position 38 is Asn, or Ala;
Xaa at position 40 is Leu, Trp, or Arg;
Xaa at position 41 is Asn, Cys, Arg, Leu, His, Met or Pro;
Xaa at position 42 is Gly, Asp, Ser, Cys, Lys, Asn, Thr, Leu, Val, Glu, Phe, Tyr, Ile, Met or Ala;
Xaa at position 43 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, Arg, Thr, Gly or Ser;
Xaa at position 44 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, Glu, Gln, or Pro;
Xaa at position 45 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, Asn, Asp, Arg, Ser, Ala, Ile or Trp;

Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Glu, Gln, His, Tyr, Ile, Val or Gly;
Xaa at position 47 is Ile, Gly, Ser, Arg, Pro, or His;
Xaa at position 48 is Leu, Ser, Cys, Arg, His, Phe, Glu, Lys, Thr, Ala, Val or Asn;
Xaa at position 49 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;
Xaa at position 50 is Glu, Leu, Thr, Asp, Asn, Ser, Ala, Ile, Val, His, Phe, Met or Tyr;
Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 52 is Asn, His, Arg, Leu, Gly, Ser, or Thr;
Xaa at position 53 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser, or;
Xaa at position 54 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn, Ala or Leu;
Xaa at position 55 is Arg, Thr, Val, Ser, Leu, or Gly;
Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Glu, Gln, Arg, His, Thr, Tyr, Phe, Leu, Val or Lys;
Xaa at position 57 is Asn or Gly;
Xaa at position 58 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
Xaa at position 59 is Glu Tyr, His, Leu;
Xaa at position 60 is Ala, Ser, Tyr, Asn, or Thr;
Xaa at position 61 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
Xaa at position 62 is Asn His, Val, Arg, Pro, Thr, or Ile;
Xaa at position 63 is Arg, Tyr, Trp, Ser, Pro, or Val;
Xaa at position 64 is Ala, Asn, Ser, or Lys;
Xaa at position 65 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 66 is Lys, Ile, Val, Asn, Glu, or Ser;
Xaa at position 67 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or His;
Xaa at position 68 is Leu, Val, Trp, Ser, Thr, or His;
Xaa at position 69 is Gln, Ala, Pro, Thr, Arg, Trp, Gly, or Leu;
Xaa at position 70 is Asn, Leu, Val, Trp, Pro, or Ala;
Xaa at position 71 is Ala, Met, Leu, Arg, Glu, Thr, Gln, Trp, or Asn;
Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;
Xaa at position 74 is Ile, Thr, Pro, Arg, Gly, Ala;

Xaa at position 75 is Glu, Lys, Asp, Pro, Trp, Ser, or Leu;

Xaa at position 76 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or Asp;

Xaa at position 77 is Ile, Ser, Arg, or Thr;

Xaa at position 78 is Leu, Ala, Ser, Glu, Gly, or Arg;

Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Ile, or Asp;

Xaa at position 80 is Asn, Trp, Val, Gly, Thr, Leu, or Arg;

Xaa at position 81 is Leu, Gln, Gly, Ala, Trp, Arg, or Lys;

Xaa at position 82 is Leu, Gln, Lys, Trp, Arg, Glu, Asn, His, Thr, Ser, Ala or Asp;

Xaa at position 83 is Pro, Thr, Trp, Arg, or Met;

Xaa at position 84 is Cys, Glu, Gly, Arg, Met, or Val;

Xaa at position 85 is Leu, Asn, or Gln;

Xaa at position 86 is Pro, Cys, Arg, Ala, or Lys;

Xaa at position 87 is Leu, Ser, Trp, or Gly;

Xaa at position 88 is Ala, Lys, Arg, Val, or Trp;

Xaa at position 89 is Thr, Asp, Cys, Leu, Val, Glu, His, or Asn;

Xaa at position 90 is Ala, Ser, Asp, Ile, or Met;

Xaa at position 91 is Ala, Ser, Thr, Phe, Leu, Asp, or His;

Xaa at position 92 is Pro, Phe, Arg, Ser, Lys, His, Gly, Ile, or Leu;

Xaa at position 93 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;

Xaa at position 94 is Arg, Ile, Ser, Glu, Leu, Val, Gln, or Ala;

Xaa at position 95 is His, Gln, Pro, Val, Leu, Thr or Asn, Lys, Ser, Ala, Trp, Phe, Ile or Tyr;

Xaa at position 96 is Pro, Lys, Tyr, Gly, Ile, or Thr;

Xaa at position 97 is Ile, Lys, Ala, or Asn;

Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr, Glu, Ser, Phe, Met, Val, Lys, Tyr or Pro;

Xaa at position 99 is Ile, Arg, Asp, Pro, Gln, Gly, Phe, or His;

Xaa at position 100 is Lys, Tyr, Leu, His, Ile, Ser, Gln, or Pro;

Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val, Tyr, Glu, Ser, Ala, Gly, Ile, Leu, or Gln;

Xaa at position 102 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;

Xaa at position 103 is Asp, or Ser;

Xaa at position 104 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu, Gln, Lys, Ala, Phe, or Gly;
Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, or His;
Xaa at position 106 is Glu, Ser, Ala, Thr, Ile, Gly, or Pro;
Xaa at position 108 is Arg, Asp, Leu, Thr, Ile, Gln, Ser, Ala or Pro;
Xaa at position 109 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;
Xaa at position 110 is Lys, Asn, Thr, Leu, Gln, His, Ser, Ala or Trp;
Xaa at position 111 is Leu, Arg, or Asp;
Xaa at position 112 is Thr, Val, Tyr, Glu, or Phe;
Xaa at position 113 is Phe, Ser, Cys, His, Gly, Asp, Lys,or Asn;
Xaa at position 114 is Tyr, Cys, His, Ser, Arg, or Leu;
Xaa at position 115 is Leu, Asn, Pro, Arg, Ala, His, Thr, or Trp,;
Xaa at position 116 is Lys, Leu, Pro, Thr, Met, Asp, Val, Trp, Ser, Asn, Arg, Ala, Tyr, Phe, Met or Ile;
Xaa at position 117 is Thr, Ser, Asn, Ile, Trp, Lys, or Pro;
Xaa at position 118 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;
Xaa at position 119 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;
Xaa at position 120 is Asn, Ala, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Asn, Lys, Asp, or Gly;
Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, Ile, Tyr, or Cys;
Xaa at position 123 is Ala, Met, Glu, His, Ser, Tyr, or Leu.

7. The human interleukin-3 mutant polypeptide of claim 1 wherein 1-15 amino acids are deleted from the C-terminus or 1-14 amino acids are deleted from the N-terminus.

8. The human interleukin-3 mutant polypeptide of claim 1 wherein;

Xaa at position 42 is Gly, Asp, Ser, Ile, Leu, Met, Tyr, or Ala;
Xaa at position 45 is Gln, Val, Met or Asn;
Xaa at position 46 is Asp, Ser, Gln, His or Val;
Xaa at position 50 is Glu or Asp;

Xaa at position 51 is Asn, Pro or Thr;
Xaa at position 62 is Asn or Pro;
Xaa at position 76 is Ser, or Pro;
Xaa at position 82 is Leu, Trp, Asp, Asn Glu, His, Phe, Ser or Tyr;
Xaa at position 95 is His, Arg, Thr, Asn or Ser;
Xaa at position 98 is His, Ile, Leu, Ala, Gln, Lys, Met, Ser, Tyr or Val;
Xaa at position 100 is Lys or Arg;
Xaa at position 101 is Asp, Pro, His, Asn, Ile or Leu;
Xaa at position 105 is Asn, or Pro;
Xaa at position 108 is Arg, Ala, or Ser;
Xaa at position 116 is Lys, Val, Trp, Ala, His, Phe, or Tyr;
Xaa at position 121 is Ala, or Ile;
Xaa at position 122 is Gln, or Ile; and
Xaa at position 123 is Ala, Met or Glu.

9. A (15-125)human interleukin-3 mutant polypeptide of the Formula V:

Asn Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
80 85 90

Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

95 100 105

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:19]
110

wherein
Xaa at position 3 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;
Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
Xaa at position 5 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;
Xaa at position 6 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;
Xaa at position 7 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn, Thr, Ser or Val;
Xaa at position 8 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln, Leu, Val, or Gly;
Xaa at position 9 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe, Leu, Ser, or Arg;
Xaa at position 10 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;
Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
Xaa at position 12 is His, Thr, Phe, Gly, Arg, Ala, or Trp;
Xaa at position 13 is Leu, Gly, Arg, Thr, Ser, or Ala;
Xaa at position 14 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;
Xaa at position 15 is Gln, Asn, Leu, Pro, Arg, or Val;
Xaa at position 16 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;
Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;
Xaa at position 18 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 19 is Pro, Leu, Gln, Ala, Thr, or Glu;
Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr, Arg, Ala, Phe, Ile or Met;
Xaa at position 21 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;
Xaa at position 22 is Asp, Leu, or Val;
Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;
Xaa at position 24 is Asn, or Ala;
Xaa at position 26 is Leu, Trp, or Arg;
Xaa at position 27 is Asn, Cys, Arg, Leu, His, Met, Pro;
Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Lys, Asn, Thr, Leu, Val, Glu, Phe, Tyr, Ile or Met;
Xaa at position 29 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, Gln,

Arg, Thr, Gly or Ser;
Xaa at position 30 is Asp, Ser, Leu, Arg, Lys, Thr,Met, Trp, Glu, Asn, Gln, Ala or Pro;
Xaa at position 31 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, Asp, Asn, Arg, Ser, Ala, Ile, Glu, His or Trp;
Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln, Lys, His, Ala, Tyr, Ile, Val or Gly;
Xaa at position 33 is Ile, Gly, Val, Ser, Arg, Pro, or His;
Xaa at position 34 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu, Lys, Thr, Ala, Met, Val or Asn;
Xaa at position 35 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;
Xaa at position 36 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser, Ala, Ile, Val, His, Phe, Met or Gln;
Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 38 is Asn, His, Arg, Leu, Gly, Ser, or Thr;
Xaa at position 39 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser, Met, or;
Xaa at position 40 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn, Lys, His, Ala or Leu;
Xaa at position 41 is Arg, Thr, Val, Ser, Leu, or Gly;
Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His, Thr, Ala, Tyr, Phe, Leu, Val or Lys;
Xaa at position 43 is Asn or Gly;
Xaa at position 44 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
Xaa at position 45 is Glu Tyr, His, Leu, Pro, or Arg;
Xaa at position 46 is Ala, Ser, Pro, Tyr, Asn, or Thr;
Xaa at position 47 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
Xaa at position 48 is Asn, His, Val, Arg, Pro, Thr, Asp, or Ile;
Xaa at position 49 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or Val;
Xaa at position 50 is Ala, Asn, Pro, Ser, or Lys;
Xaa at position 51 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 53 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or His;
Xaa at position 54 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or Leu;
Xaa at position 56 is Asn, Leu, Val, Trp, Pro, or Ala;

Xaa at position 57 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln, Trp, or Asn;

Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;

Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;

Xaa at position 60 is Ile, Met, Thr, Pro, Arg, Gly, Ala;

Xaa at position 61 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser, Gln, or Leu;

Xaa at position 62 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or Asp;

Xaa at position 63 is Ile, Ser, Arg, Thr, or Leu;

Xaa at position 64 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;

Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or Asp;

Xaa at position 66 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;

Xaa at position 67 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;

Xaa at position 68 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn, His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;

Xaa at position 69 is Pro, Ala, Thr, Trp, Arg, or Met;

Xaa at position 70 is Cys, Glu, Gly, Arg, Met, or Val;

Xaa at position 71 is Leu, Asn, Val, or Gln;

Xaa at position 72 is Pro, Cys, Arg, Ala, or Lys;

Xaa at position 73 is Leu, Ser, Trp, or Gly;

Xaa at position 74 is Ala, Lys, Arg, Val, or Trp;

Xaa at position 75 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn, or Ser;

Xaa at position 76 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or Met;

Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;

Xaa at position 78 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile or Leu;

Xaa at position 79 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;

Xaa at position 80 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys, His, Ala or Pro;

Xaa at position 81 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr, Asn, Lys, Ser, Ala, Trp, Phe, Ile or Tyr;

Xaa at position 82 is Pro, Lys, Tyr, Gly, Ile, or Thr;

Xaa at position 83 is Ile, Val, Lys, Ala, or Asn;

Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr, Glu, Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;

Xaa at position 85 is Ile, Leu, Arg, Asp, Val, Pro, Gln, Gly, Ser, Phe, or His;
Xaa at position 86 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln, Pro;
Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val, Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu or Gln;
Xaa at position 88 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
Xaa at position 89 is Asp, or Ser;
Xaa at position 90 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu, Gln, Lys, Ala, Phe, or Gly;
Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
Xaa at position 92 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;
Xaa at position 94 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His, Ser, Ala, or Pro;
Xaa at position 95 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;
Xaa at position 96 is Lys, Asn, Thr, Leu, Gln, Arg, His, Glu, Ser, Ala or Trp;
Xaa at position 97 is Leu, Ile, Arg, Asp, or Met;
Xaa at position 98 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;
Xaa at position 99 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp, Lys, Leu, Ile, Val or Asn;
Xaa at position 100 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;
Xaa at position 101 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr, Trp, or Met;
Xaa at position 102 is Lys, Leu, Pro, Thr, Met, Asp, Val, Glu, Arg, Trp, Ser, Asn, His, Ala, Tyr, Phe, Gln, or Ile;
Xaa at position 103 is Thr, Ser, Asn, Ile, Trp, Lys, or Pro;
Xaa at position 104 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;
Xaa at position 105 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;
Xaa at position 106 is Asn, Ala, Pro, Leu, His, Val, or Gln;
Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or Gly;
Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- or Met-Ala- preceding the

amino acid in position 1; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding native amino acids of (1-133) human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

10. A (15-125)human interleukin-3 mutant polypeptide of the Formula VI:

Asn Cys Xaa Xaa Xaa Xaa Xaa Glu Xaa Xaa Xaa Xaa Leu Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa Xaa Glu Xaa
20 25 30

Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Cys Xaa Pro Xaa Xaa Xaa
65 70 75

Xaa Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa
80 85 90

Xaa Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Xaa
95 100 105

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:20]
110

wherein
Xaa at position 3 is Ser, Gly, Asp, Met, or Gln;
Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
Xaa at position 5 is Met, Phe, Ile, Arg, or Ala;
Xaa at position 6 is Ile or Pro;

Xaa at position 7 is Asp, or Glu;
Xaa at position 9 is Ile, Val, Ala, Leu, or Gly;
Xaa at position 10 is Ile, Val, Phe, or Leu;
Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
Xaa at position 12 is His, Phe, Gly, Arg, or Ala;
Xaa at position 14 is Lys, Leu, Gln, Gly, Pro, or Val;
Xaa at position 15 is Gln, Asn, Leu, Arg, or Val;
Xaa at position 16 is Pro, His, Thr, Gly, or Gln;
Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;
Xaa at position 18 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 19 is Pro, Leu, Gln, Ala, or Glu;
Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln, Glu, Ile, Phe, Thr or Met;
Xaa at position 21 is Leu, Ala, Asn, Pro, Gln, or Val;
Xaa at position 22 is Asp or Leu;
Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;
Xaa at position 24 is Asn or Ala;
Xaa at position 27 is Asn, Cys, Arg, His, Met, or Pro;
Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu, Met, Tyr, or Arg;
Xaa at position 30 is Asp, or Glu;
Xaa at position 31 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn Glu, Ser or Trp;
Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln, Glu, His, Ile, Lys, Tyr, Val or Gly;
Xaa at position 33 is Ile, Val, or His;
Xaa at position 35 is Met, Asn, or Asp;
Xaa at position 36 is Glu, Thr, Ala, Asn, Ser or Asp;
Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 38 is Asn or Gly;
Xaa at position 39 is Leu, Met, or Phe;
Xaa at position 40 is Arg, Ala or Ser;
Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;
Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn, Glu, His, Leu, Thr, Val or Lys;
Xaa at position 45 is Glu, Tyr, His, Leu, or Arg;
Xaa at position 46 is Ala, Ser, Asn, or Thr;
Xaa at position 47 is Phe or Ser;

Xaa at position 48 is Asn, Val, Pro, Thr, or Ile;
Xaa at position 49 is Arg, Tyr, Lys, Ser, His, or Val;
Xaa at position 50 is Ala or Asn;
Xaa at position 51 is Val, Thr, Leu, or Ser;
Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 53 is Ser, Phe, Val, Gly, Asn, Ile, or His;
Xaa at position 54 is Leu, Val, Ile, Phe, or His;
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
Xaa at position 56 is Asn or Pro;
Xaa at position 57 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;
Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or Pro;
Xaa at position 60 is Ile or Met;
Xaa at position 61 is Glu, Gly, Asp, Ser, or Gln;
Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or Asp;
Xaa at position 63 is Ile, Ser, or Leu;
Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or Asp;
Xaa at position 66 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 67 is Leu, or Val;
Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 69 is Pro, Ala, Thr, Trp, or Met;
Xaa at position 71 is Leu or Val;
Xaa at position 73 is Leu or Ser;
Xaa at position 74 is Ala, Arg, or Trp;
Xaa at position 75 is Thr, Asp, Glu, His, Asn, or Ser;
Xaa at position 76 is Ala, Asp, or Met;
Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;
Xaa at position 78 is Pro or Ser;
Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile, Phe, Ser or Thr;
Xaa at position 82 is Pro or Tyr;
Xaa at position 83 is Ile, Val, or Ala;
Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr,

Arg, Gln, Glu, Lys, Met, Ser, Tyr, Val or Pro;
Xaa at position 85 is Ile, Leu, Val, or Phe;
Xaa at position 86 is Lys, Leu, His, Arg, Ile, Gln, Pro or Ser;
Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val, Asn, Ile, Leu or Tyr;
Xaa at position 88 is Gly, Glu, Lys, or Ser;
Xaa at position 90 is Trp, Val, Tyr, Met, or Leu;
Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
Xaa at position 92 is Glu, Ser, Ala, or Gly;
Xaa at position 94 is Arg, Ala, Gln, Ser or Lys;
Xaa at position 95 is Arg, Thr, Glu, Leu, Ser, or Gly;
Xaa at position 98 is Thr, Val, Gln, Glu, His, or Ser;
Xaa at position 100 is Tyr or Trp;
Xaa at position 101 is Leu or Ala;
Xaa at position 102 is Lys, Thr, Met, Val, Trp, Ser, Leu, Ala, Asn, Gln, His, Met, Phe, Tyr or Ile;
Xaa at position 103 is Thr, Ser, or Asn;
Xaa at position 105 is Glu, Ser, Pro, Leu, Thr, or Tyr;
Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or Gly;
Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- or Met-Ala- preceding the amino acid in position 1; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

11. A (15-125)human interleukin-3 mutant polypeptide according to Claim 6 of the Formula VII:

Asn Cys Xaa Xaa Xaa Ile Xaa Glu Xaa Xaa Xaa Xaa Leu Lys Xaa

1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa Xaa Asn Leu Asn Xaa Glu Xaa
20 25 30

Xaa Xaa Ile Leu Met Xaa Xaa Asn Leu Xaa Xaa Xaa Asn Leu Glu
35 40 45

Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Ile
50 55 60

Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Cys Xaa Pro Xaa Xaa Thr
65 70 75

Ala Xaa Pro Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Asp Xaa
80 85 90

Xaa Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Glu
95 100 105

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:21]
110

wherein
Xaa at position 3 is Ser, Gly, Asp, Met, or Gln;
Xaa at position 4 is Asn, His, or Ile;
Xaa at position 5 is Met or Ile;
Xaa at position 7 is Asp or Glu;
Xaa at position 9 is Ile, Ala, Leu, or Gly;
Xaa at position 10 is Ile, Val, or Leu;
Xaa at position 11 is Thr, His, Gln, or Ala;
Xaa at position 12 is His or Ala;
Xaa at position 15 is Gln, Asn, or Val;
Xaa at position 16 is Pro, Gly, or Gln;
Xaa at position 17 is Pro, Asp, Gly, or Gln;
Xaa at position 18 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 19 is Pro or Glu;
Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Ala, Arg,

Gln, Glu, Ile, Phe, Thr or Met;
Xaa at position 21 is Leu, Ala, Asn, Pro, Gln, or Val;
Xaa at position 23 is Phe, Ser, Pro, or Trp;
Xaa at position 24 is Asn or Ala;
Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu, Met Tyr or Arg;
Xaa at position 30 is Asp or Glu;
Xaa at position 31 is Gln, Val, Met, Leu, Thr, Ala, Asn, Glu, Ser or Lys;
Xaa at position 32 is Asp, Phe, Ser, Thr, Ala, Asn, Gln, Glu, His, Ile, Lys, Tyr, Val or Cys;
Xaa at position 36 is Glu, Ala, Asn, Ser or Asp;
Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 40 is Arg or Ala;
Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;
Xaa at position 42 is Pro, Gly, Ser, Gln, Ala, Arg, Asn, Glu, Leu, Thr, Val or Lys;
Xaa at position 46 is Ala or Ser;
Xaa at position 48 is Asn, Pro, Thr, or Ile;
Xaa at position 49 is Arg or Lys;
Xaa at position 50 is Ala or Asn;
Xaa at position 51 is Val or Thr;
Xaa at position 52 is Lys or Arg;
Xaa at position 53 is Ser, Phe, or His;
Xaa at position 54 is Leu, Ile, Phe, or His;
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
Xaa at position 57 is Ala, Pro, or Arg;
Xaa at position 58 is Ser, Glu, Arg, or Asp;
Xaa at position 59 is Ala or Leu;
Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, or Gly;
Xaa at position 63 is Ile or Leu;
Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or Asp;
Xaa at position 66 is Asn, Gly, Glu, or Arg;
Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His, Ile, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 69 is Pro or Thr;
Xaa at position 71 is Leu or Val;

Xaa at position 73 is Leu or Ser;
Xaa at position 74 is Ala or Trp;
Xaa at position 77 is Ala or Pro;
Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Phe, Ser or Thr;
Xaa at position 82 is Pro or Tyr;
Xaa at position 83 is Ile or Val;
Xaa at position 84 is His, Ile, Asn, Leu, Ala, Thr, Leu, Arg, Gln, Leu, Lys, Met, Ser, Tyr, Val or Pro;
Xaa at position 85 is Ile, Leu, or Val;
Xaa at position 86 is Lys, Arg, Ile, Gln, Pro, or Ser;
Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Asn, Ile, Leu or Tyr;
Xaa at position 90 is Trp or Leu;
Xaa at position 91 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
Xaa at position 92 is Glu, or Gly;
Xaa at position 94 is Arg, Ala, or Ser;
Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;
Xaa at position 98 is Thr, Val, or Gln;
Xaa at position 100 is Tyr or Trp;
Xaa at position 101 is Leu or Ala;
Xaa at position 102 is Lys, Thr, Val, Trp, Ser, Ala, His, Met, Phe, Tyr or Ile;
Xaa at position 103 is Thr or Ser;
Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;
Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

which can additionally have Met- or Met-Ala- preceding the amino acid in position 1; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (15-125)human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

12. A (15-125)human interleukin-3 mutant polypeptide according to Claim 7 of the Formula VIII:

Asn Cys Xaa Xaa Met Ile Asp Glu Xaa Ile Xaa Xaa Leu Lys Xaa
1 5 10 15

Xaa Pro Xaa Pro Xaa Xaa Asp Phe Xaa Asn Leu Asn Xaa Glu Asp
20 25 30

Xaa Xaa Ile Leu Met Xaa Xaa Asn Leu Arg Xaa Xaa Asn Leu Glu
35 40 45

Ala Phe Xaa Arg Xaa Xaa Lys Xaa Xaa Xaa Asn Ala Ser Ala Ile
50 55 60

Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Cys Leu Pro Xaa Xaa Thr
65 70 75

Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Xaa Xaa Xaa Gly Asp Trp
80 85 90

Xaa Glu Phe Xaa Xaa Lys Leu Xaa Phe Tyr Leu Xaa Xaa Leu Glu
95 100 105

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:22]
110

wherein
Xaa at position 3 is Ser, Gly, Asp, or Gln;
Xaa at position 4 is Asn, His, or Ile;
Xaa at position 9 is Ile, Ala, Leu, or Gly;
Xaa at position 11 is Thr, His, or Gln;
Xaa at position 12 is His or Ala;
Xaa at position 15 is Gln or Asn;
Xaa at position 16 is Pro or Gly;
Xaa at position 18 is Leu, Arg, Asn, or Ala;
Xaa at position 20 is Leu, Val, Ser, Ala, Arg, Gln, Glu, Ile,

Phe, Thr or Met;
Xaa at position 21 is Leu, Ala, Asn, or Pro;
Xaa at position 24 is Asn or Ala;
Xaa at position 28 is Gly, Asp, Ser, Ala, Asn, Ile, Leu, Met, Tyr or Arg;
Xaa at position 31 is Gln, Val, Met, Leu, Ala, Asn, Glu or Lys;
Xaa at position 32 is Asp, Phe, Ser, Ala, Gln, Glu, His, Val or Thr;
Xaa at position 36 is Glu, Asn, Ser or Asp;
Xaa at position 37 is Asn, Arg, Pro, Thr, or His;
Xaa at position 41 is Arg, Leu, or Gly;
Xaa at position 42 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;
Xaa at position 48 is Asn, Pro, or Thr;
Xaa at position 50 is Ala or Asn;
Xaa at position 51 is Val or Thr;
Xaa at position 53 is Ser or Phe;
Xaa at position 54 is Leu or Phe;
Xaa at position 55 is Gln, Ala, Glu, or Arg;
Xaa at position 62 is Ser, Val, Asn, Pro, or Gly;
Xaa at position 63 is Ile or Leu;
Xaa at position 65 is Lys, Asn, Met, Arg, Ile, or Gly;
Xaa at position 66 is Asn, Gly, Glu, or Arg;
Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 73 is Leu or Ser;
Xaa at position 74 is Ala or Trp;
Xaa at position 77 is Ala or Pro;
Xaa at position 79 is Thr, Asp, or Ala;
Xaa at position 81 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;
Xaa at position 84 is His, Ile, Asn, Ala, Thr, Arg, Gln, Glu, Lys, Met, Ser, Tyr, Val or Leu;
Xaa at position 85 is Ile or Leu;
Xaa at position 86 is Lys or Arg;
Xaa at position 87 is Asp, Pro, Met, Lys, His, Pro, Asn, Ile, Leu or Tyr;
Xaa at position 91 is Asn, Pro, Ser, Ile or Asp;
Xaa at position 94 is Arg, Ala, or Ser;
Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;

Xaa at position 98 is Thr or Gln;
Xaa at position 102 is Lys, Val, Trp, or Ile;
Xaa at position 103 is Thr, Ala, His, Phe, Tyr or Ser;
Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 107 is Ala, Ser, Ile, Pro, or Asp;
Xaa at position 108 is Gln, Met, Trp, Phe, Pro, His, Ile, or Tyr;
Xaa at position 109 is Ala, Met, Glu, Ser, or Leu;

and which can additionally have Met- or Met-Ala- preceding the amino acid in position 1; and wherein from one to three of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133)human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

13. A (15-125) human interleukin-3 mutant polypeptide according to claim 12 wherein said polypeptide has Ala at position 50.

14. A (15-125) human interleukin-3 mutant polypeptide according to claim 9 with the proviso that when Xaa at position 22 is Leu, and/or Xaa at position 34 is Gly or Glu, and/or Xaa at position 44 is Ala, and/or Xaa at position 46 is Lys or Ala, and/or Xaa at position 50 is Lys, and/or Xaa at position 59 is Pro or Arg, and/or Xaa at position 63 is Lys, and/or Xaa at position 75 is Gly or Arg, and/or Xaa at position 94 is Pro, and/or Xaa at position 98 is Arg, and/or Xaa at position 106 is Lys, and/or Xaa at position 110 is Ala or Glu, and/or Xaa at position 111 is Met, then there must be at least one additional substitution besides the ones indicated.

15. A (15-125) human interleukin-3 mutant polypeptide of claim 9 wherein:
Xaa at position 17 is Ser, Lys, Asp, Met, Gln, or Arg;
Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
Xaa at position 19 is Met, Arg, Gly, Ala, or Cys;
Xaa at position 20 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;
Xaa at position 21 is Asp, Phe, Lys, Arg, Ala, Gly, or Val;

Xaa at position 22 is Glu, Trp, Pro, Ser, Ala, His, or Gly;
Xaa at position 23 is Ile, Ala, Gly, Trp, Lys, Leu, Ser, or Arg;
Xaa at position 24 is Ile, Gly, Arg, or Ser;
Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
Xaa at position 26 is His, Thr, Phe, Gly, Ala, or Trp;
Xaa at position 27 is Leu, Gly, Arg, Thr, Ser, or Ala;
Xaa at position 28 is Lys, Leu, Gln, Gly, Pro, Val or Trp;
Xaa at position 29 is Gln, Asn, Loh, Pro, Arg, or Val;
Xaa at position 30 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;
Xaa at position 31 is Pro, Asp, Gly, Arg, Leu, or Gln;
Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
Xaa at position 33 is Pro, Leu, Gln, Thr, or Glu;
Xaa at position 34 is Leu, Gly, Ser, or Lys;
Xaa at position 35 is Leu, Ala, Gly, Asn, Pro, or Gln;
Xaa at position 36 is Asp, Leu, or Val;
Xaa at position 37 is Phe, Ser, or Pro;
Xaa at position 38 is Asn, or Ala;
Xaa at position 40 is Leu, Trp, or Arg;
Xaa at position 41 is Asn, Cys, Arg, Leu, His, Met, Pro;
Xaa at position 42 is Gly, Asp, Ser, Cys, or Ala;
Xaa at position 42 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, or Ser;
Xaa at position 44 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, or Pro;
Xaa at position 45 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, or Trp;
Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, or Gly;
Xaa at position 47 is Ile, Gly, Ser, Arg, Pro, or His;
Xaa at position 48 is Leu, Ser, Cys, Arg, His, Phe, or Asn;
Xaa at position 49 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;
Xaa at position 50 is Glu, Leu, Thr, Asp, or Tyr;
Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
Xaa at position 52 is Asn, His, Arg, Leu, Gly, Ser, or Thr;
Xaa at position 53 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser, or;
Xaa at position 54 is Arg, Asp, Ile, Ser, Val, Thr, Gln, or Leu;

Xaa at position 55 is Arg, Thr, Val, Ser, Leu, or Gly;
Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, or Lys;
Xaa at position 57 is Asn or Gly;
Xaa at position 58 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
Xaa at position 59 is Glu Tyr, His, Leu, Pro, or Arg;
Xaa at position 60 is Ala, Ser, Tyr, Asn, or Thr;
Xaa at position 61 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
Xaa at position 62 is Asn His, Val, Arg, Pro, Thr, or Ile;
Xaa at position 63 is Arg, Tyr, Trp, Ser, Pro, or Val;
Xaa at position 64 is Ala, Asn, Ser, or Lys;
Xaa at position 65 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 66 is Lys, Ile, Val, Asn, Glu, or Ser;
Xaa at position 67 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or His;
Xaa at position 68 is Leu, Val, Trp, Ser, Thr, or His;
Xaa at position 69 is Gln, Ala, Pro, Thr, Arg, Trp, Gly, or Leu;
Xaa at position 70 is Asn, Leu, Val, Trp, Pro, or Ala;
Xaa at position 71 is Ala, Met, Leu, Arg, Glu, Thr, Gln, Trp, or Asn;
Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;
Xaa at position 74 is Ile, Thr, Pro, Arg, Gly, Ala;
Xaa at position 75 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser, or Leu;
Xaa at position 76 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or Asp;
Xaa at position 77 is Ile, Ser, Arg, or Thr;
Xaa at position 78 is Leu, Ala, Ser, Glu, Gly, or Arg;
Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Ile, or Asp;
Xaa at position 80 is Asn, Trp, Val, Gly, Thr, Leu, or Arg;
Xaa at position 81 is Leu, Gln, Gly, Ala, Trp, Arg, or Lys;
Xaa at position 82 is Leu, Gln, Lys, Trp, Arg, or Asp;
Xaa at position 83 is Pro, Thr, Trp, Arg, or Met;
Xaa at position 84 is Cys, Glu, Gly, Arg, Met, or Val;
Xaa at position 85 is Leu, Asn, or Gln;
Xaa at position 86 is Pro, Cys, Arg, Ala, or Lys;

Xaa at position 87 is Leu, Ser, Trp, or Gly;
Xaa at position 88 is Ala, Lys, Arg, Val, or Trp;
Xaa at position 89 is Thr, Asp, Cys, Leu, Val, Glu, His, or Asn;
Xaa at position 90 is Ala, Ser, Asp, Ile, or Met;
Xaa at position 91 is Ala, Ser, Thr, Phe, Leu, Asp, or His;
Xaa at position 92 is Pro, Phe, Arg, Ser, Lys, His, or Leu;
Xaa at position 93 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;
Xaa at position 94 is Arg, Ile, Ser, Glu, Leu, Val, or Pro;
Xaa at position 95 is His, Gln, Pro, Val, Leu, Thr or Tyr;
Xaa at position 96 is Pro, Lys, Tyr, Gly, Ile, or Thr;
Xaa at position 97 is Ile, Lys, Ala, or Asn;
Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr, or Pro;
Xaa at position 99 is Ile, Arg, Asp, Pro, Gln, Gly, Phe, or His;
Xaa at position 100 is Lys, Tyr, Leu, His, Ile, Ser, Gln, or Pro;
Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val, Tyr, or Gln;
Xaa at position 102 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
Xaa at position 103 is Asp, or Ser;
Xaa at position 104 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu, Gln, Lys, Ala, Phe, or Gly;
Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, or His;
Xaa at position 106 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;
Xaa at position 108 is Arg, Asp, Leu, Thr, Ile, or Pro;
Xaa at position 109 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly.

16. The human interleukin-3 mutant polypetide of claim 9: wherein;

Xaa at position 28 is Gly, Asp, Ser, Ile, Leu, Met, Tyr, or Ala;
Xaa at position 31 is Gln, Val, Met or Asn;
Xaa at position 32 is Asp, Ser, Ala, Gln, His or Val;
Xaa at position 36 is Glu or Asp;
Xaa at position 37 is Asn, Pro or Thr;
Xaa at position 48 is Asn or Pro;

Xaa at position 62 is Ser, or Pro;
Xaa at position 68 is Leu, Trp, Asp, Asn Glu, His, Phe, Ser or Tyr;
Xaa at position 81 is His, Arg, Thr, Asn or Ser;
Xaa at position 84 is His, Ile, Leu, Ala, Arg, Gln, Lys, Met, Ser, Tyr or Val;
Xaa at position 86 is Lys or Arg;
Xaa at position 87 is Asp, Pro, His, Asn, Ile or Leu;
Xaa at position 91 is Asn, or Pro;
Xaa at position 94 is Arg, Ala, or Ser;
Xaa at position 102 is Lys, Val, Trp, Ala, His, Phe, or Tyr;
Xaa at position 107 is Ala, or Ile;
Xaa at position 108 is Gln, or Ile; and
Xaa at position 109 is Ala, Met or Glu.

17. A (15-125) human interleukin-3 mutant polypeptide according to claim 15 with the proviso that when Xaa at position 34 is Gly, and/or Xaa at position 59 is Pro or Arg and/or Xaa at position 75 is Gly or Arg, and/or Xaa at position 94 is Pro, and/or Xaa at position 106 is Lys and/or Xaa at position 110 in Ala, then there must be at least one additional substitution besides the ones indicated.

18. A mutant human interleukin-3 polypeptide according to claim 5 which is selected from the group consisting of
a polypeptide having an amino acid sequence corresponding to SEQ ID NO:66;
a polypeptide having an amino acid sequence corresponding to SEQ ID NO:67; and
a polypeptide having an amino acid sequence corresponding to SEQ ID NO:69.

19. A pharmaceutical composition for the treatment of hematopoietic cell deficiencies comprising a therapeutically effective amount of a mutant human interleukin-3 polypeptide selected from the group consisting of a polypeptide of claim 1, a polypeptide of claim 2, a polypeptide of claim 3, a polypeptide of claim 4, a polypeptide of claim 5, a polypeptide of claim 6, a polypeptide of claim 7, a polypeptide of claim 8, a polypeptide of claim 9, a polypeptide of claim 10, a polypeptide of claim 11, a

polypeptide of claim 12, a polypeptide of claim 13, a polypeptide of claim 14, a polypeptide of claim 15, a polypeptide of claim 16, a polypeptide of claim 17; a polypeptide of claim 18 and a pharmaceutically acceptable carrier.

20. A pharmaceutical composition according to Claim 18 for the treatment of hematopoietic cell deficiencies comprising a therapeutically effective amount of a mutant human interleukin-3 polypeptide selected from the group consisting of

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:66;

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:67; and

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:69.

21. A method of stimulating the production of hematopoietic cells which comprises administering a therapeutically effective amount of a mutant human interleukin-3 polypeptide selected from the group consisting of a polypeptide of claim 1, a polypeptide of claim 2, a polypeptide of claim 3, a polypeptide of claim 4, a polypeptide of claim 5, a polypeptide of claim 6, a polypeptide of claim 7, a polypeptide of claim 8, a polypeptide of claim 9, a polypeptide of claim 10, a polypeptide of claim 11, a polypeptide of claim 12, a polypeptide of claim 13, a polypeptide of claim 14, a polypeptide of claim 14, a polypeptide of claim 15, a polypeptide of claim 16, a polypeptide of claim 17, and a polypeptide of claim 18 to a patient in need of such treatment.

22. A method according to claim 21 of stimulating the production of hematopoietic cells which comprises administering a therapeutically effective amount of a mutant human interleukin-3 polypeptide selected from the group consisting of

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:66; and

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:67.

23. A recombinant DNA sequence comprising vector DNA and a DNA that encodes a mutant human interleukin-3 polypeptide having the amino acid sequence of a polypeptide selected from the group consisting of a polypeptide of claim 1, a polypeptide of claim 2, a polypeptide of claim 3, a polypeptide of claim 4, a polypeptide of claim 5, a polypeptide of claim 6, a polypeptide of claim 7, a polypeptide of claim 8, a polypeptide of claim 9, a polypeptide of claim 10, a polypeptide of claim 11, a polypeptide of claim 12, a polypeptide of claim 13, a polypeptide of claim 14, a polypeptide of claim 15, a polypeptide of claim 16, a polypeptide of claim 17 and a polypeptide of claim 18.

24. A recombinant DNA sequence according to Claim 23 that encodes a mutant human interleukin-3 polypeptide selected from the group consisting of

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:66; and

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:67.

25. A host cell containing a recombinant DNA sequence comprising vector DNA and a DNA that encodes a mutant human interleukin-3 polypeptide having the amino acid sequence of a polypeptide selected from the group consisting of a polypeptide of claim 1, a polypeptide of claim 2, a polypeptide of claim 3, a polypeptide of claim 4, a polypeptide of claim 5, a polypeptide of claim 6, a polypeptide of claim 7, a polypeptide of claim 8, a polypeptide of claim 9, and a polypeptide of claim 10, a polypeptide of claim 11, a polypeptide of claim 12, a polypeptide of claim 13, a polypeptide of claim 14, a polypeptide of claim 15, a polypeptide of claim 16, and a polypeptide of claim 17, a polypeptide of claim 18, and capable of expressing the encoded polypeptide.

26. A host cell according to claim 25 containing a recombinant DNA sequence that encodes a mutant human interleukin-3 polypeptide selected from the group consisting of

a polypeptide having an amino acid sequence corresponding to

SEQ ID NO:66;
a polypeptide having an amino acid sequence corresponding to SEQ ID NO:67; and
a polypeptide having an amino acid sequence corresponding to SEQ ID NO:69.

27. A method of producing a mutant human interleukin-3 polypeptide comprising the steps of:

(a) culturing a host cell containing a recombinant DNA sequence comprising vector DNA and a DNA sequence that encodes a mutant human interleukin-3 polypeptide having the amino acid sequence of a polypeptide selected from the group consisting of a polypeptide of claim 1, a polypeptide of claim 2, a polypeptide of claim 3, a polypeptide of claim 4, a polypeptide of claim 5, a polypeptide of claim 6, a polypeptide of claim 7, a polypeptide of claim 8, a polypeptide of claim 9, a polypeptide of claim 10, a polypeptide of claim 11, a polypeptide of claim 12, a polypeptide of claim 13, a polypeptide of claim 14, a polypeptide of claim 14, a polypeptide of claim 15, a polypeptide of claim 16, and a polypeptide of claim 17, a polypeptide of claim 18 and capable of expressing the encoded polypeptide under conditions permitting expression of the recombinant DNA; and

(b) harvesting the polypeptide from the culture.

28. A method of producing a mutant human interleukin-3 polypeptide comprising the steps of:

(a) culturing a host cell containing a recombinant DNA sequence comprising vector DNA and a DNA sequence that encodes a mutant human interleukin-3 polypeptide selected from the group consisting of
a polypeptide having an amino acid sequence corresponding to SEQ ID NO:66; and
a polypeptide having an amino acid sequence corresponding to SEQ ID NO:67; and capable of expressing the encoded polypeptide under conditions permitting expression of the

recombinant DNA; and

(b) harvesting the polypeptide from the culture.

29. A vector containing a gene having a DNA sequence that encodes a mutant human interleukin-3 polypeptide having the amino acid sequence of a polypeptide selected from the group consisting of a polypeptide of claim 1, a polypeptide of claim 2, a polypeptide of claim 3, a polypeptide of claim 4, a polypeptide of claim 5, a polypeptide of claim 6, a polypeptide of claim 7, a polypeptide of claim 8, a polypeptide of claim 9, a polypeptide of claim 10, a polypeptide of claim 11, a polypeptide of claim 12, a polypeptide of claim 13, a polypeptide of claim 14, a polypeptide of claim 14, a polypeptide of claim 15, a polypeptide of claim 16, a polypeptide of claim 17 and a polypeptide of claim 18.

30. A recombinant DNA vector comprising a promoter, a ribosome binding site, and a signal peptide directly linked to a DNA sequence encoding a mutant human interleukin-3 polypeptide selected from the group consisting of a polypeptide of claim 1, a polypeptide of claim 2, a polypeptide of claim 3, a polypeptide of claim 4, a polypeptide of claim 5, a polypeptide of claim 6, a polypeptide of claim 7, a polypeptide of claim 8, a polypeptide of claim 9, a polypeptide of claim 10, a polypeptide of claim 11, a polypeptide of claim 12, a polypeptide of claim 13, a polypeptide of claim 14, a polypeptide of claim 14, a polypeptide of claim 15, a polypeptide of claim 16, a polypeptide of claim 17, and a polypeptide of claim 18, said vector being capable of directing expression of said mutant human interleukin-3 polypeptide.

31. A recombinant DNA vector comprising a promoter, a ribosome binding site, and a signal peptide directly linked to a DNA sequence encoding a mutant human interleukin-3 polypeptide selected from the group consisting of

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:66;

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:67; and

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:69; said vector being capable of directing expression of said mutant human interleukin-3 polypeptide.

32. A recombinant DNA vector according to Claim 30 wherein the promoter is AraBAD or recA.

33. A recombinant DNA vector according to Claim 30 wherein the ribosome binding site is g10-L.

34. A recombinant DNA vector according to Claim 30 wherein the signal peptide is a lamB signal peptide.

35. A recombinant DNA vector according to Claim 30 wherein the signal peptide is the lamB signal peptide depicted in Figure 8.

36. A recombinant DNA vector according to Claim 30 wherein the promoter is AraBAD or recA and the ribosome binding site is g10-L.

37. A recombinant DNA vector according to Claim 30 wherein the promoter is AraBAD, the ribosome binding site is g10-L, and the signal peptide is a lamB signal peptide.

38. A recombinant DNA vector according to Claim 30 wherein the promoter is AraBAD, the ribosome binding site is g10-L, and the signal peptide is the lamB signal peptide depicted in Figure 8.

39. A recombinant bacterial host which comprises the vector of Claim 30 wherein said host secretes a mutant human interleukin-3 polypeptide selected from the group consisting of

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:66;

a polypeptide having an amino acid sequence corresponding to

SEQ ID NO:67; and

a polypeptide having an amino acid sequence corresponding to SEQ ID NO:69

```
     1                   5                  10
ATG GCT CCA ATG ACT CAG ACT ACT TCT CTT AAG ACT TCT
Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser

        15                  20                  25
TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA
Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr

                30                  35
CAC TTA AAG CAG CCA CCT TTG CCT TTG CTG GAC TTC AAC
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn

    40                  45                  50
AAC CTC AAT GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn

            55                  60
AAC CTT CGA AGG CCA AAC CTG GAG GCA TTC AAC AGG GCT
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala

65                  70                  75
GTC AAG AGT TTA CAG AAT GCA TCA GCA ATT GAG AGC ATT
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile

        80                  85                  90
CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG GCC
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala

                95                  100
GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp

    105                 110                 115
TGG AAT GAA TTC CGT CGT AAA CTG ACC TTC TAT CTG AAA
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys

            120                 125
ACC TTG GAG AAC GCG CAG GCT CAA CAG ACC ACT CTG TCG
Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser

130
CTA GCG ATC TTT TAA TAA  [SEQ ID NO:144]
Leu Ala Ile Phe END END  [SEQ ID NO:128]
```

FIG. 1

```
                C
                l
                a
                I
aa20   ATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTGCTGGACTTCAACAAC
    1  ---------+---------+---------+---------+---------+---------+ 60
       IleAspGluIleIleThrHisLeuLysGlnProProLeuProLeuLeuAspPheAsnAsn -

                          E
                          c                                   X
                          o                                   h
                          R                                   o
                          V                                   I
       CTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCCAAACCTCGAG
   61  ---------+---------+---------+---------+---------+---------+ 120
       LeuAsnGlyGluAspGlnAspIleLeuMetGluAsnAsnLeuArgArgProAsnLeuGlu -

                                    P       N
                                    s       s
                                    t       i
                                    I       I
       GCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCAT [SEQ ID NO:145] aa70
  121  ---------+---------+---------+------- 157
       AlaPheAsnArgAlaValLysSerLeuGlnAsnAla  [SEQ ID NO:146]
```

FIG. 2: ClaI to NsiI Replacement Fragment

FIG. 2

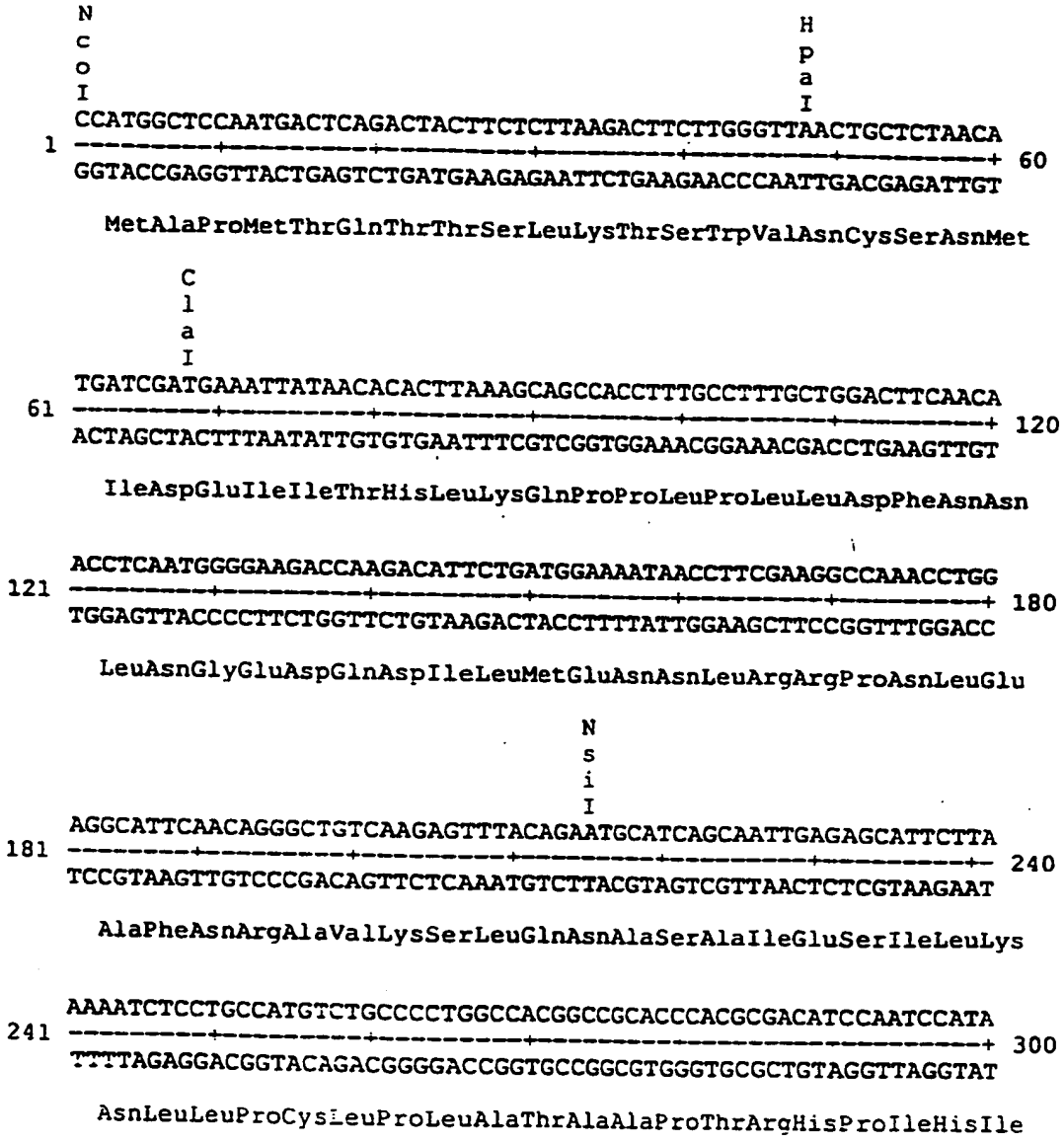
NcoI
HpaI
CCATGGCTCCAATGACTCAGACTACTTCTCTTAAGACTTCTTGGGTTAACTGCTCTAACA
1
GGTACCGAGGTTACTGAGTCTGATGAAGAGAATTCTGAAGAACCCAATTGACGAGATTGT
60
MetAlaProMetThrGlnThrThrSerLeuLysThrSerTrpValAsnCysSerAsnMet
ClaI
TGATCGATGAAATTATAACACACTTAAAGCAGCCACCTTTGCCTTTGCTGGACTTCAACA
61
ACTAGCTACTTTAATATTGTGTGAATTTCGTCGGTGGAAACGGAAACGACCTGAAGTTGT
120
IleAspGluIleIleThrHisLeuLysGlnProProLeuProLeuLeuAspPheAsnAsn
ACCTCAATGGGGAAGACCAAGACATTCTGATGGAAAATAACCTTCGAAGGCCAAACCTGG
121
TGGAGTTACCCCTTCTGGTTCTGTAAGACTACCTTTTATTGGAAGCTTCCGGTTTGGACC
180
LeuAsnGlyGluAspGlnAspIleLeuMetGluAsnAsnLeuArgArgProAsnLeuGlu
NsiI
AGGCATTCAACAGGGCTGTCAAGAGTTTACAGAATGCATCAGCAATTGAGAGCATTCTTA
181
TCCGTAAGTTGTCCCGACAGTTCTCAAATGTCTTACGTAGTCGTTAACTCTCGTAAGAAT
240
AlaPheAsnArgAlaValLysSerLeuGlnAsnAlaSerAlaIleGluSerIleLeuLys
AAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATA
241
TTTTAGAGGACGGTACAGACGGGGACCGGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTAT
300
AsnLeuLeuProCysLeuProLeuAlaThrAlaAlaProThrArgHisProIleHisIle

FIG. 3A

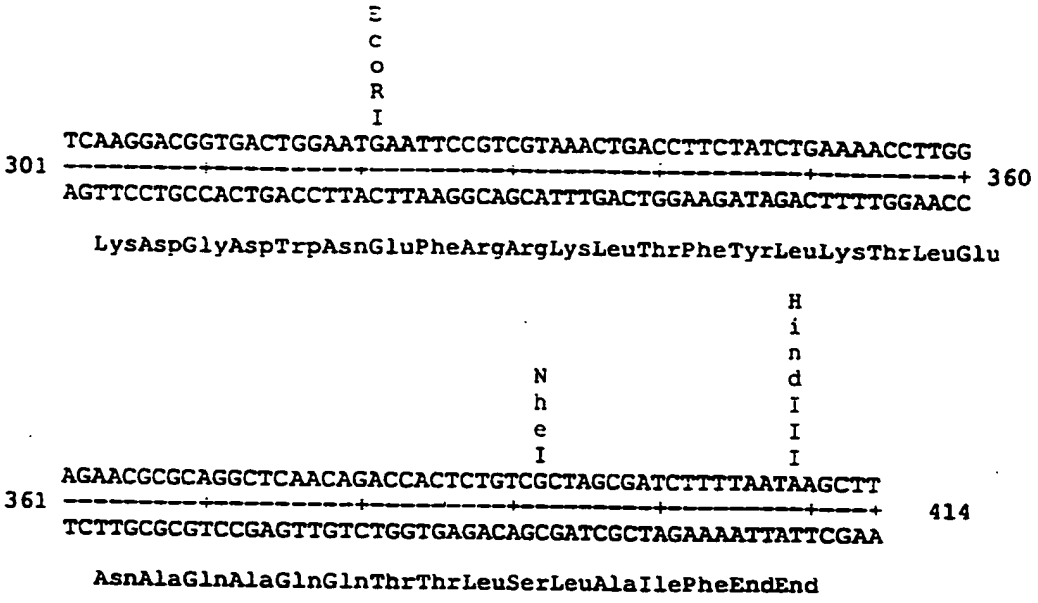
EcoRI
TCAAGGACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGG
301
AGTTCCTGCCACTGACCTTACTTAAGGCAGCATTTGACTGGAAGATAGACTTTTGGAACC
360
LysAspGlyAspTrpAsnGluPheArgArgLysLeuThrPheTyrLeuLysThrLeuGlu
NheI
HindIII
AGAACGCGCAGGCTCAACAGACCACTCTGTCGCTAGCGATCTTTTAATAAGCTT
361
TCTTGCGCGTCCGAGTTGTCTGGTGAGACAGCGATCGCTAGAAAATTATTCGAA
414
AsnAlaGlnAlaGlnGlnThrThrLeuSerLeuAlaIlePheEndEnd

FIG. 3B

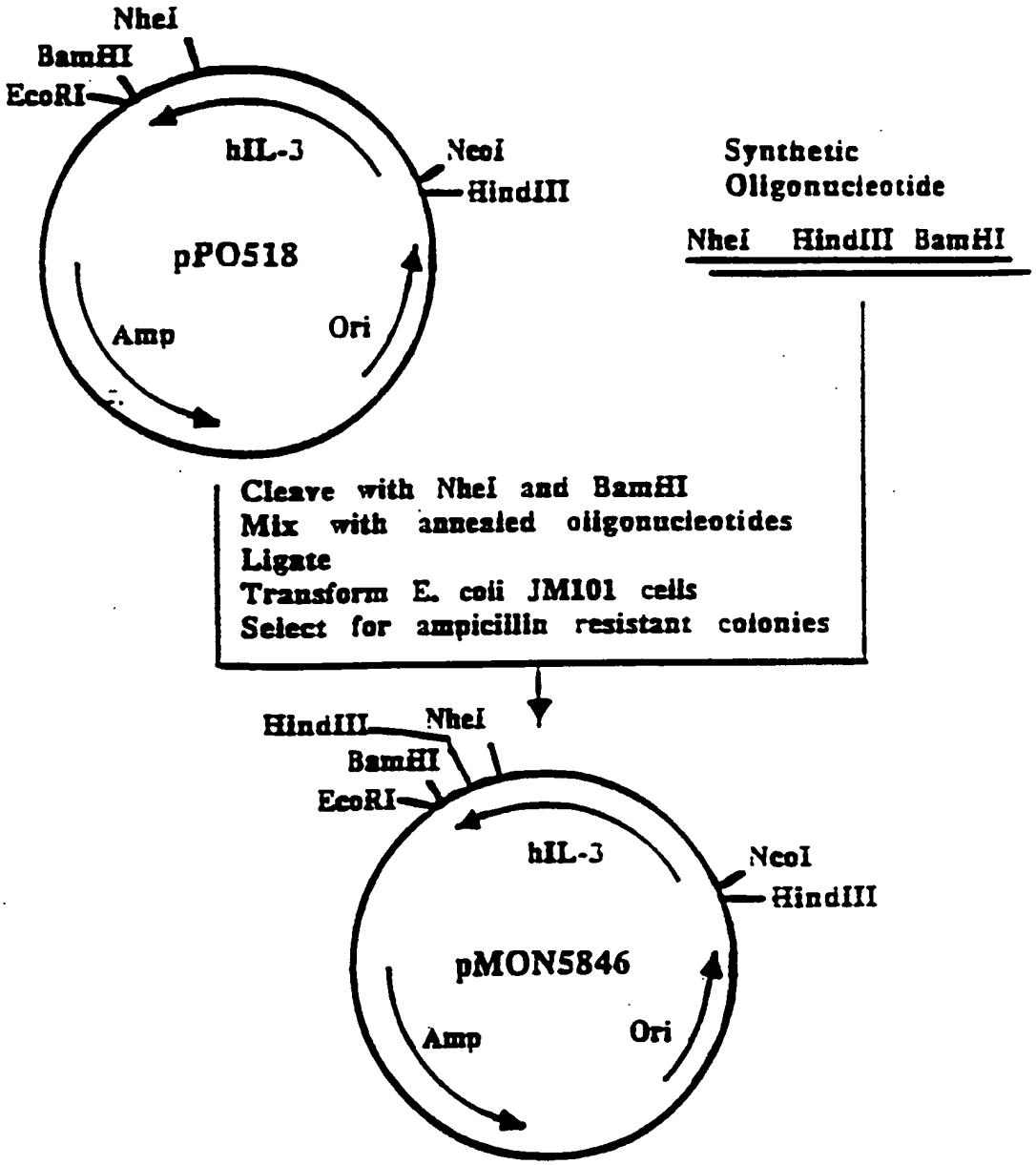
NheI
BamHI
EcoRI
hIL-3
NcoI
HindIII
pPO518
Amp
Ori
Synthetic
Oligonucleotide
NheI HindIII BamHI
Cleave with NheI and BamHI
Mix with annealed oligonucleotides
Ligate
Transform E. coli JM101 cells
Select for ampicillin resistant colonies
HindIII
NheI
BamHI
EcoRI
hIL-3
NcoI
HindIII
pMON5846
Amp
Ori

FIG. 4

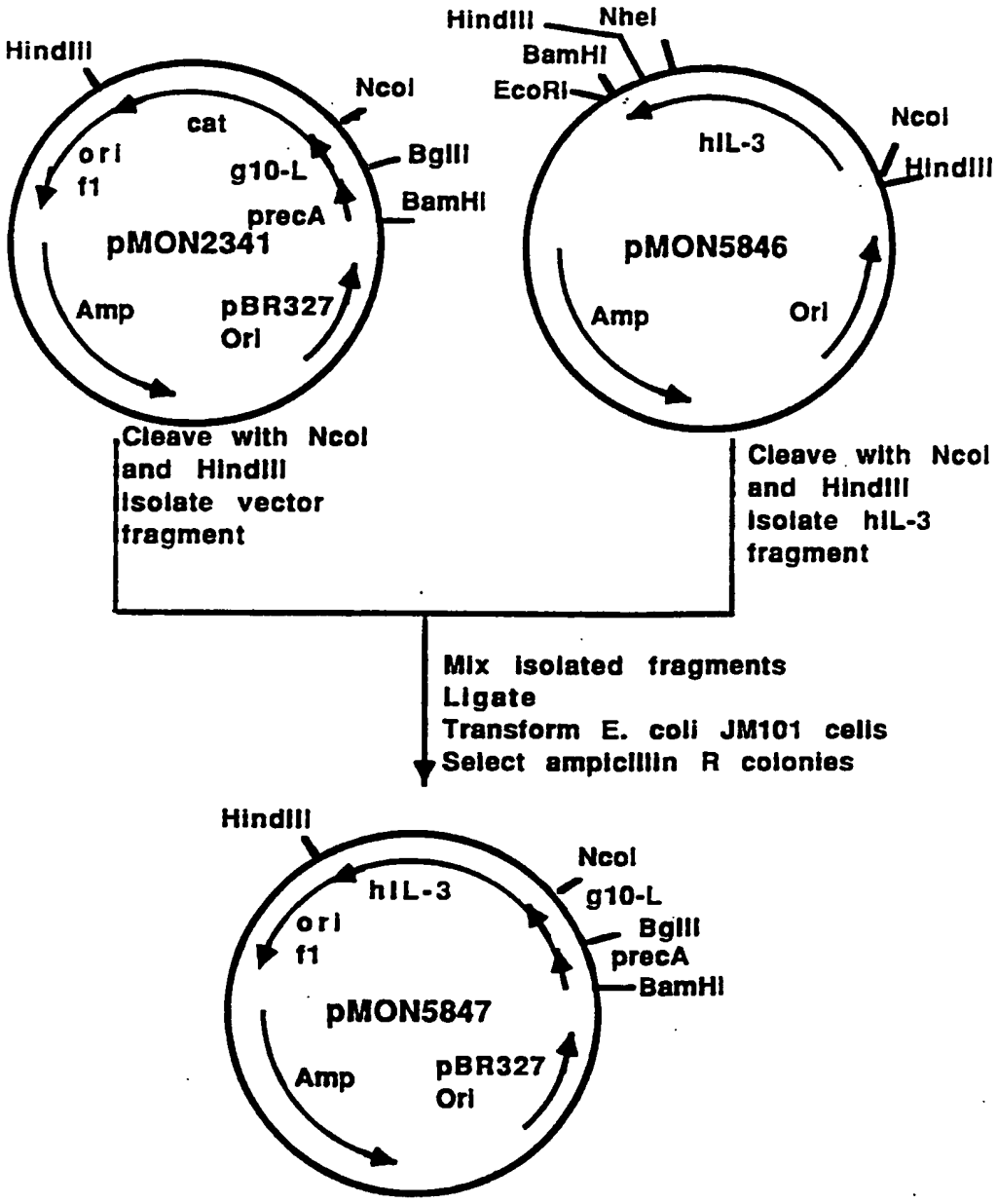
HindIII
NcoI
cat
ori
f1
g10-L
BglII
precA
BamHI
pMON2341
Amp
pBR327
Ori
HindIII
NheI
BamHI
EcoRI
NcoI
hIL-3
HindIII
pMON5846
Amp
Ori
Cleave with NcoI and HindIII Isolate vector fragment
Cleave with NcoI and HindIII Isolate hIL-3 fragment
Mix isolated fragments
Ligate
Transform E. coli JM101 cells
Select ampicillin R colonies
HindIII
NcoI
hIL-3
g10-L
ori
f1
BglII
precA
BamHI
pMON5847
Amp
pBR327
Ori

FIG. 5

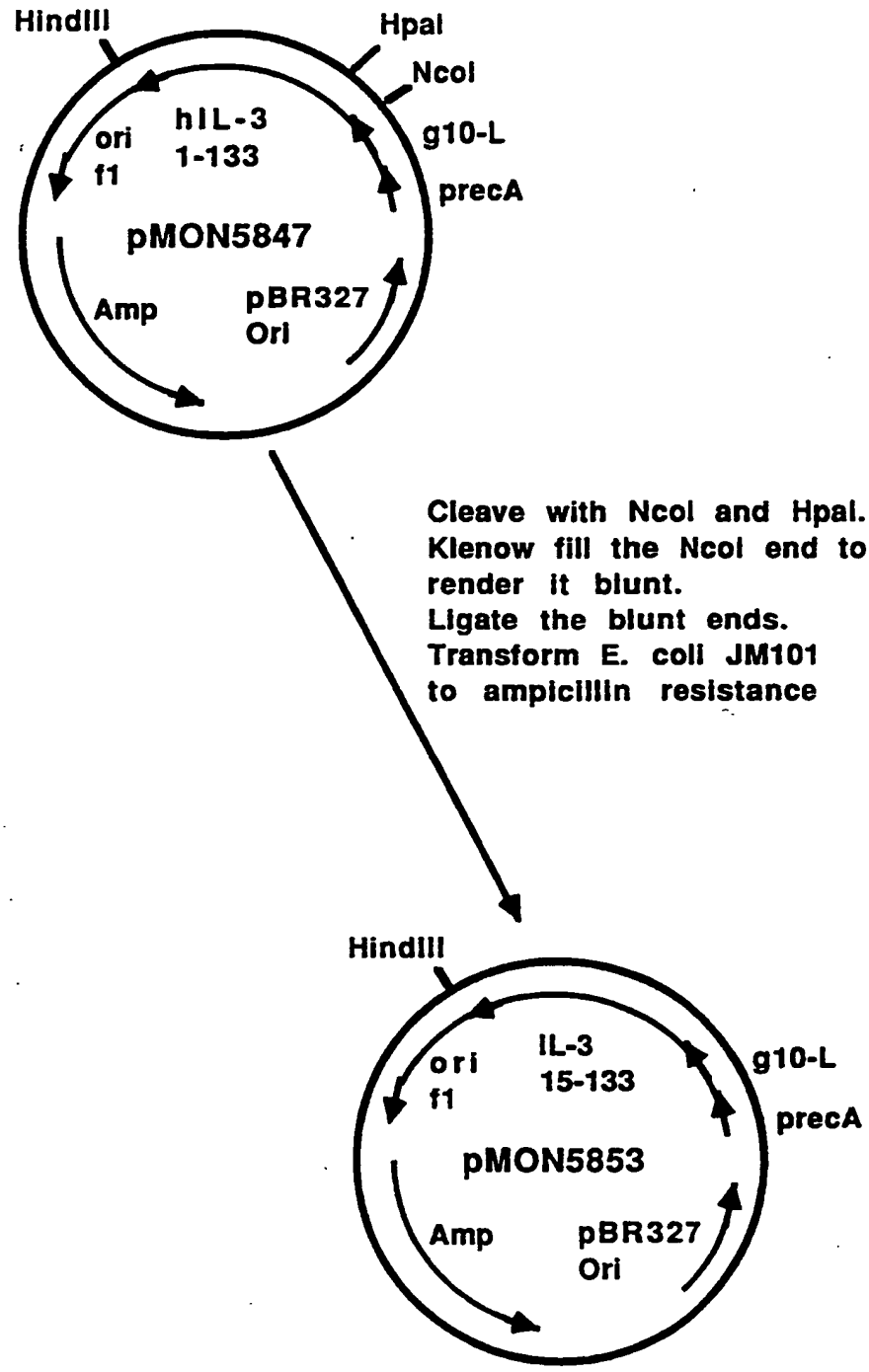
HindIII
HpaI
NcoI
hIL-3
1-133
ori
f1
g10-L
precA
pMON5847
Amp
pBR327
Ori
Cleave with NcoI and HpaI.
Klenow fill the NcoI end to
render it blunt.
Ligate the blunt ends.
Transform E. coli JM101
to ampicillin resistance
HindIII
IL-3
15-133
ori
f1
g10-L
precA
pMON5853
Amp
pBR327
Ori

FIG. 6

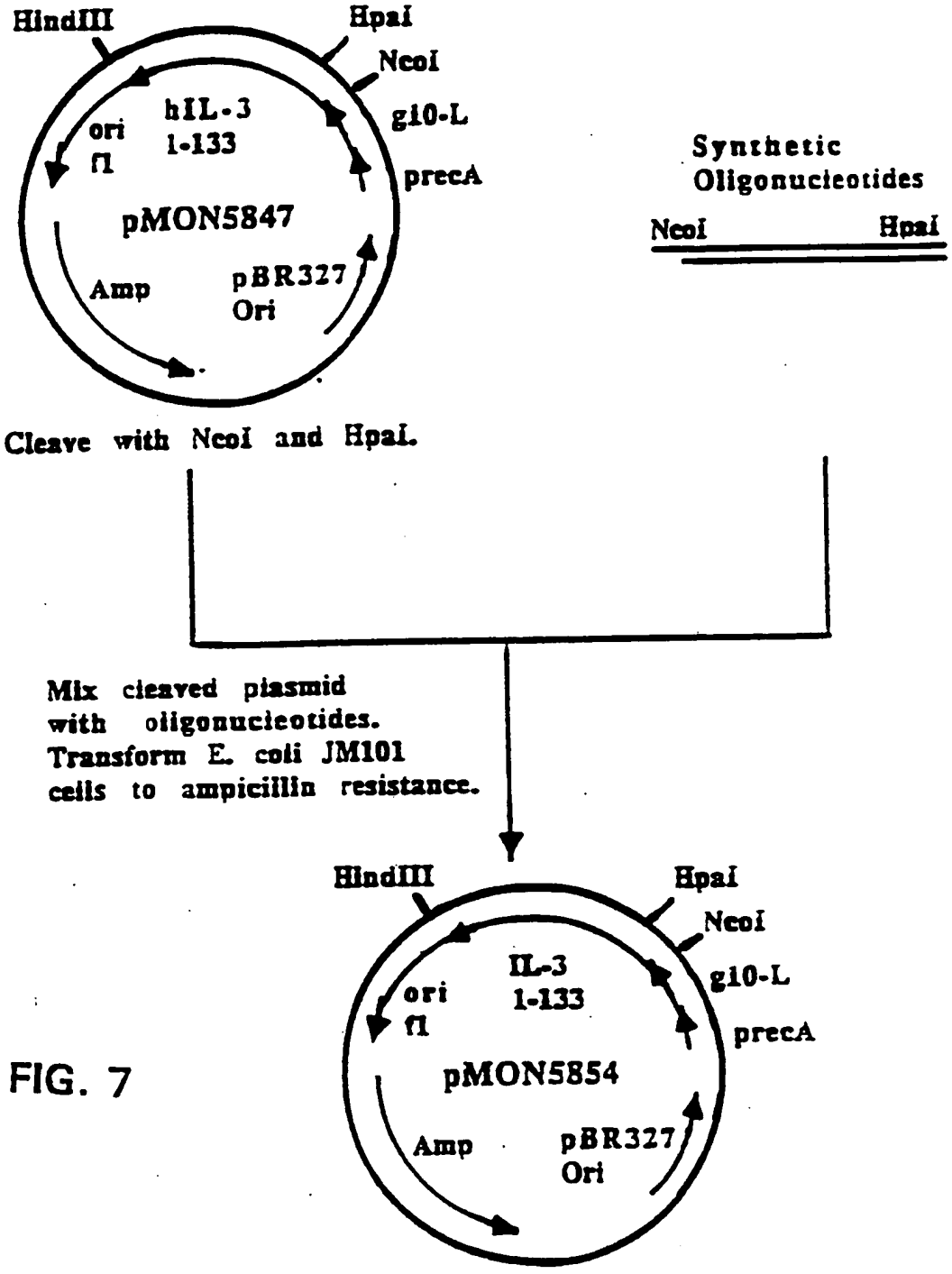
HindIII
HpaI
NcoI
hIL-3
1-133
gi0-L
ori
f1
precA
pMON5847
Amp
pBR327
Ori
Synthetic
Oligonucleotides
NcoI
HpaI
Cleave with NcoI and HpaI.
Mix cleaved plasmid
with oligonucleotides.
Transform E. coli JM101
cells to ampicillin resistance.
HindIII
HpaI
NcoI
IL-3
1-133
gi0-L
ori
f1
precA
pMON5854
Amp
pBR327
Ori

FIG. 7

```
   ATGATGATTACTCTGCGCAAACTTCCTCTGGCGGTTGCCGTCGCAGCGGGCGTAATGTCT
1  ---------+---------+---------+---------+---------+---------+ 60
   TACTACTAATGAGACGCGTTTGAAGGAGACCGCCAACGGCAGCGTCGCCCGCATTACAGA

   MetMetIleThrLeuArgLysLeuProLeuAlaValAlaValAlaAlaGlyValMetSer

          N
          c
          o
          I
   GCTCAGGCCATGGCTAACTGC      [SEQ ID NO: 149]
61 ---------+---------+- 81
   CGAGTCCGGTACCGATTGACG      [SEQ ID NO: 150]

   AlaGlnAlaMetAlaAsnCys      [SEQ ID NO: 14]
                    ↑
```

lamB Signal Peptide

FIG. 8

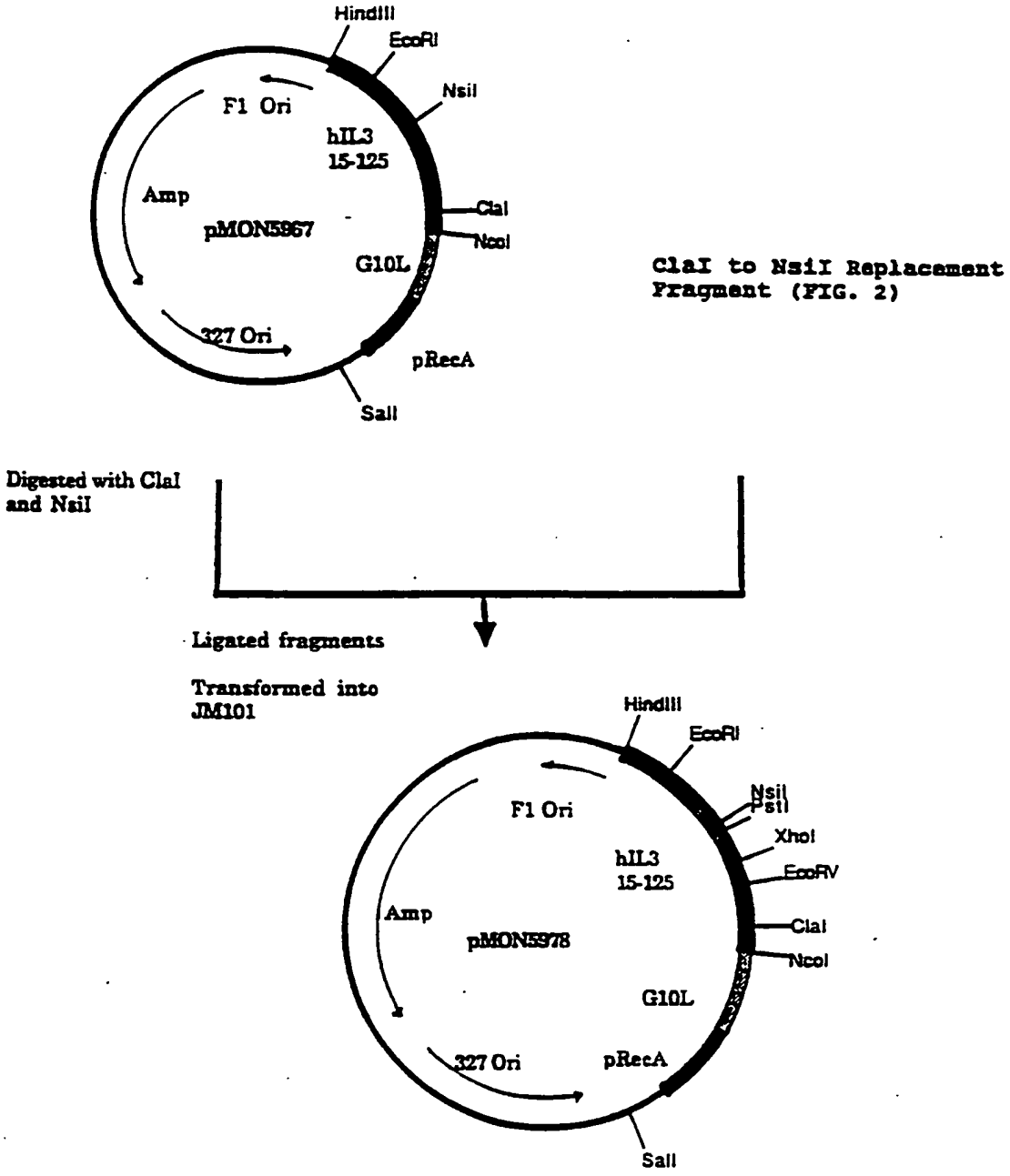
HindIII
EcoRI
NsiI
F1 Ori
hIL3
15-125
Amp
ClaI
pMON5967
NcoI
G10L
327 Ori
pRecA
SalI
ClaI to NsiI Replacement Fragment (FIG. 2)
Digested with ClaI and NsiI
Ligated fragments
Transformed into JM101
HindIII
EcoRI
NsiI
PstI
F1 Ori
XhoI
hIL3
15-125
EcoRV
Amp
ClaI
pMON5978
NcoI
G10L
327 Ori
pRecA
SalI

FIG. 9

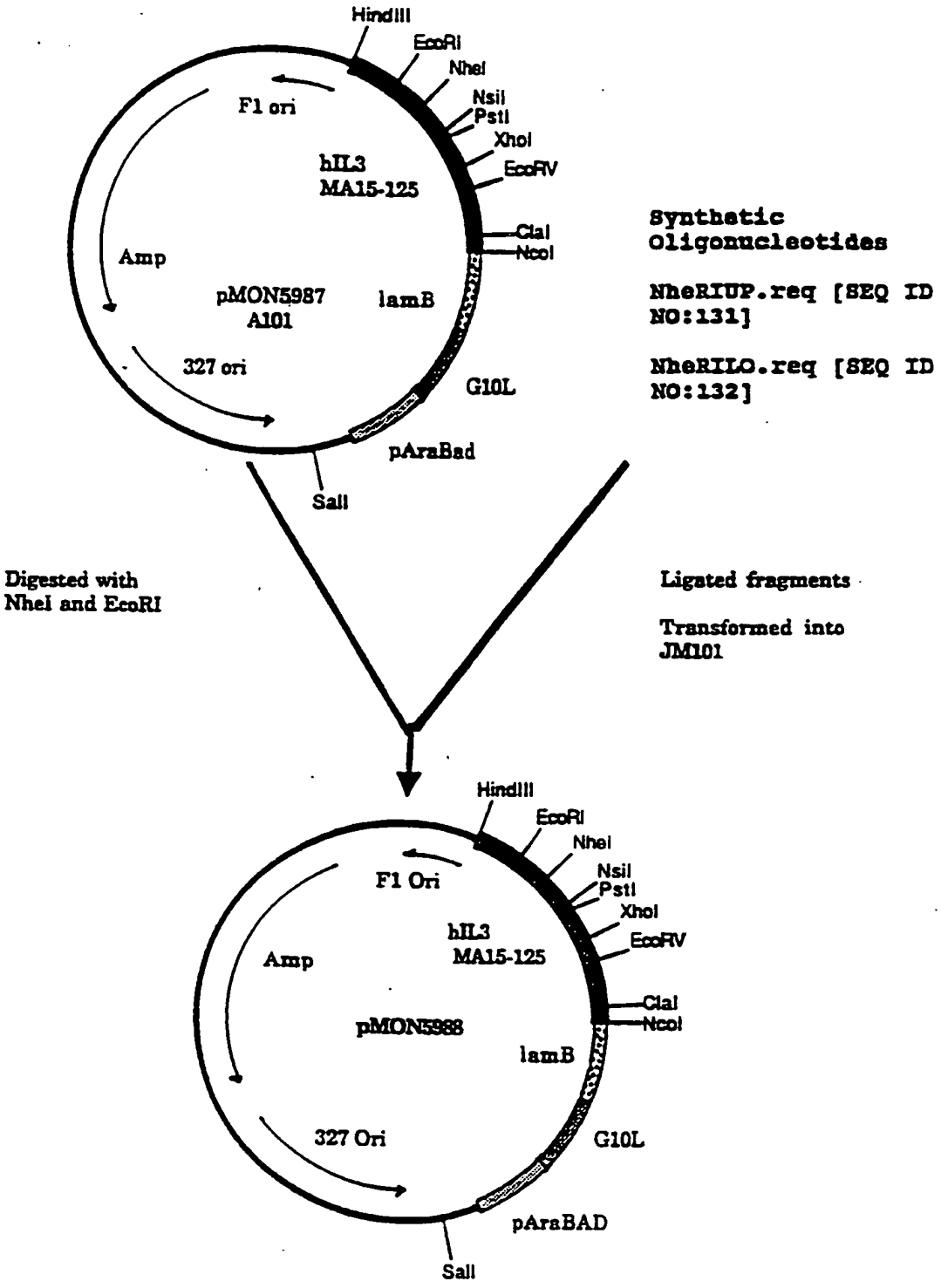
HindIII
EcoRI
NheI
NsiI
PstI
XhoI
EcoRV
ClaI
NcoI
F1 ori
hIL3
MA15-125
Amp
pMON5987
A101
lamB
327 ori
G10L
pAraBad
SalI
Synthetic Oligonucleotides
NheRIUP.req [SEQ ID NO:131]
NheRILO.req [SEQ ID NO:132]
Digested with NheI and EcoRI
Ligated fragments
Transformed into JM101
HindIII
EcoRI
NheI
NsiI
PstI
XhoI
EcoRV
ClaI
NcoI
F1 Ori
hIL3
MA15-125
Amp
pMON5988
lamB
327 Ori
G10L
pAraBAD
SalI

FIG 10

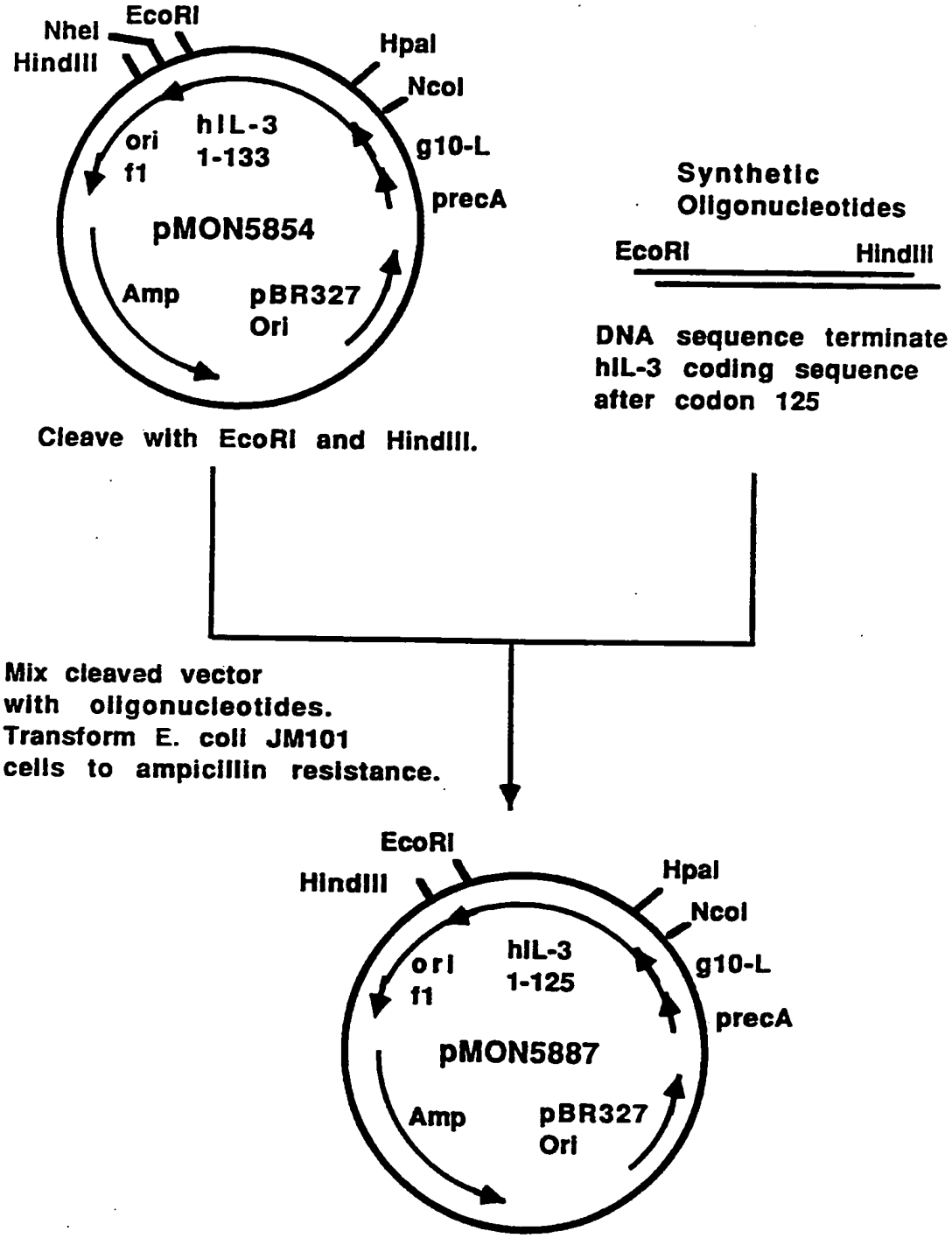
NheI
EcoRI
HindIII
HpaI
NcoI
ori
f1
hIL-3
1-133
g10-L
precA
pMON5854
Amp
pBR327
Ori
Synthetic
Oligonucleotides
EcoRI
HindIII
DNA sequence terminate
hIL-3 coding sequence
after codon 125
Cleave with EcoRI and HindIII.
Mix cleaved vector
with oligonucleotides.
Transform E. coli JM101
cells to ampicillin resistance.
EcoRI
HindIII
HpaI
NcoI
ori
f1
hIL-3
1-125
g10-L
precA
pMON5887
Amp
pBR327
Ori

FIG. 11

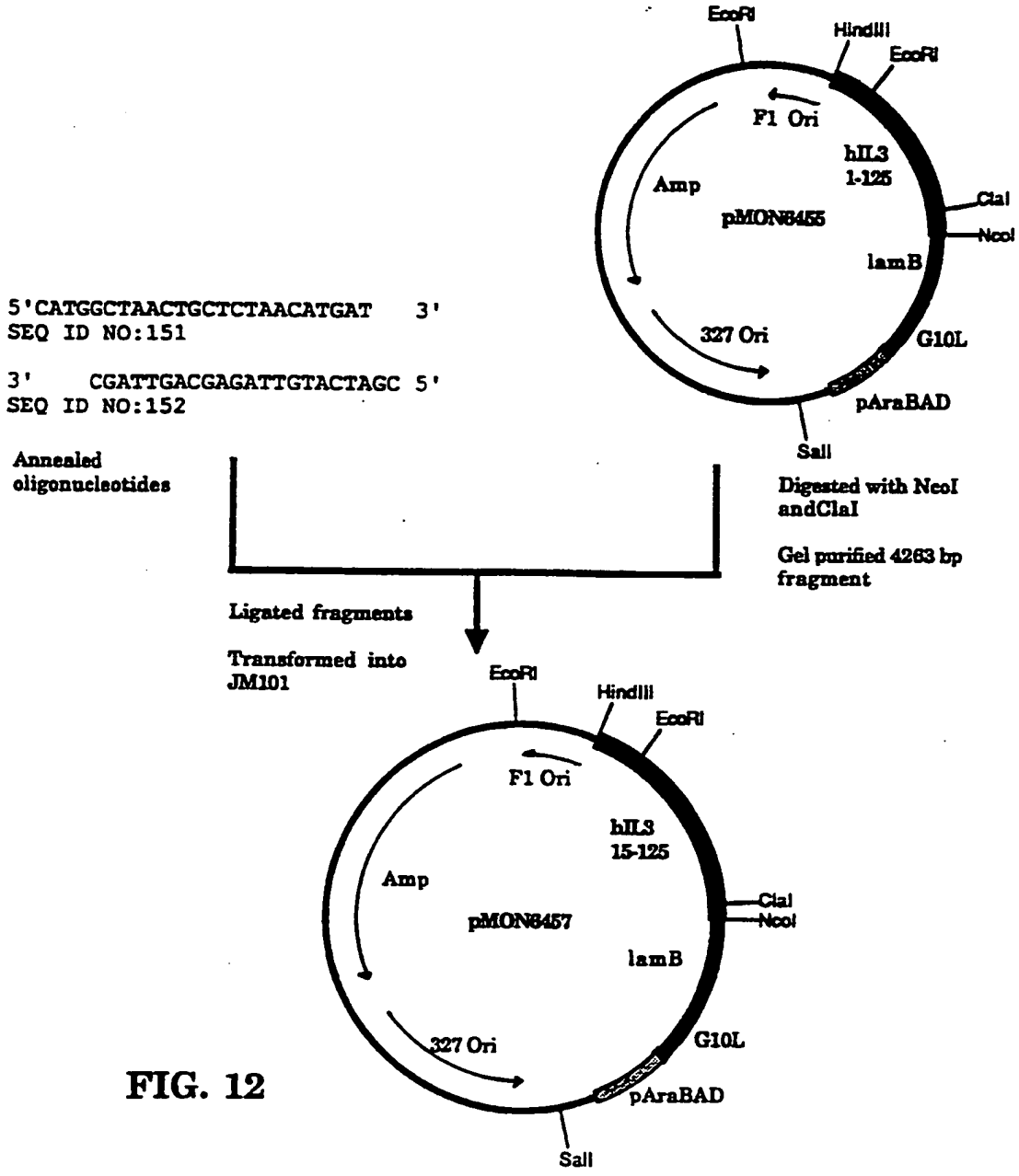
5'CATGGCTAACTGCTCTAACATGAT 3'
SEQ ID NO:151
3' CGATTGACGAGATTGTACTAGC 5'
SEQ ID NO:152
Annealed oligonucleotides
EcoRI
HindIII
EcoRI
F1 Ori
hIL3 1-125
Amp
ClaI
NcoI
pMON8455
lamB
327 Ori
G10L
pAraBAD
SalI
Digested with NcoI andClaI
Gel purified 4263 bp fragment
Ligated fragments
Transformed into JM101
EcoRI
HindIII
EcoRI
F1 Ori
hIL3 15-125
Amp
ClaI
NcoI
pMON8457
lamB
327 Ori
G10L
pAraBAD
SalI

FIG. 12

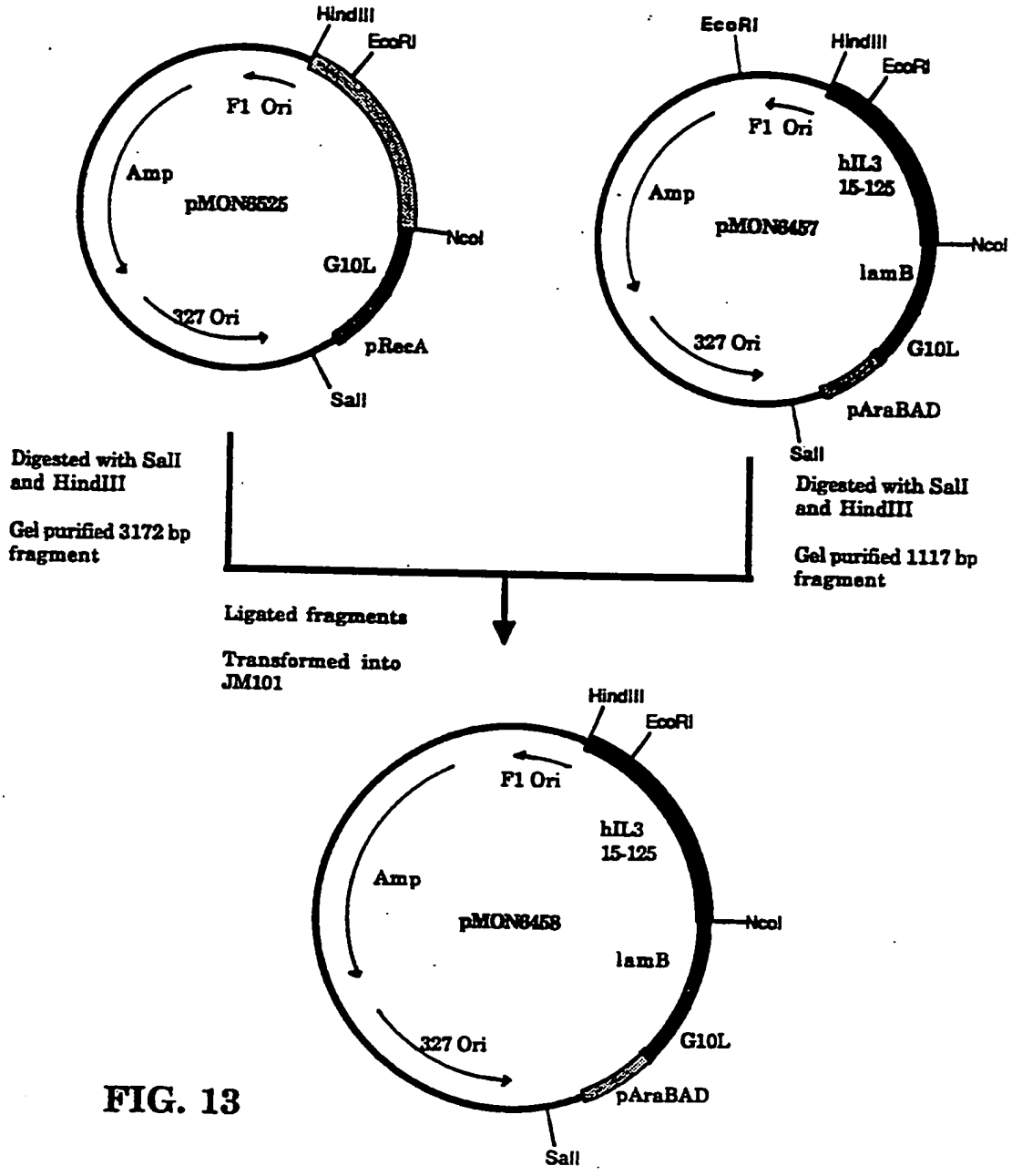
HindIII
EcoRI
F1 Ori
Amp
pMON8525
NcoI
G10L
327 Ori
pRecA
SalI
EcoRI
HindIII
EcoRI
F1 Ori
hIL3
15-125
Amp
pMON8457
NcoI
lamB
327 Ori
G10L
pAraBAD
SalI
Digested with SalI and HindIII
Gel purified 3172 bp fragment
Digested with SalI and HindIII
Gel purified 1117 bp fragment
Ligated fragments
Transformed into JM101
HindIII
EcoRI
F1 Ori
hIL3
15-125
Amp
pMON8458
NcoI
lamB
327 Ori
G10L
pAraBAD
SalI

FIG. 13

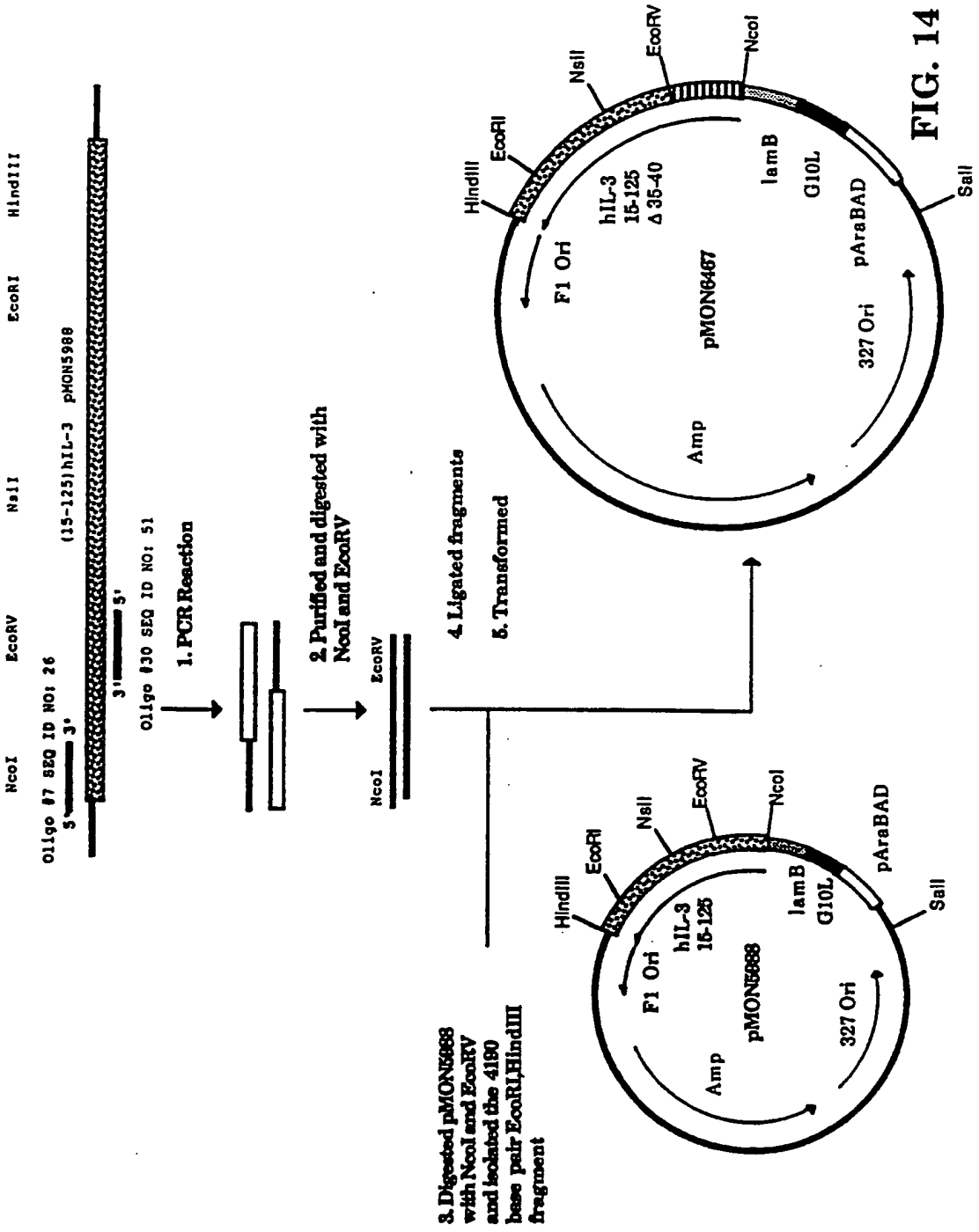
NcoI
EcoRV
NsiI
EcoRI
HindIII
Oligo #7 SEQ ID NO: 26
5'
3'
(15-125)hIL-3
pMON5988
3'
5'
Oligo #30 SEQ ID NO: 51
1. PCR Reaction
2. Purified and digested with NcoI and EcoRV
NcoI
EcoRV
4. Ligated fragments
5. Transformed
3. Digested pMON5988 with NcoI and EcoRV and isolated the 4180 base pair EcoRI,HindIII fragment
HindIII
EcoRI
NsiI
EcoRV
NcoI
pAraBAD
SalI
F1 Ori
hIL-3
15-125
Δ 35-40
pMON6467
lamB
G10L
327 Ori
Amp
HindIII
EcoRI
NsiI
EcoRV
NcoI
pAraBAD
SalI
F1 Ori
hIL-3
15-125
pMON5988
lamB
G10L
327 Ori
Amp

FIG. 14

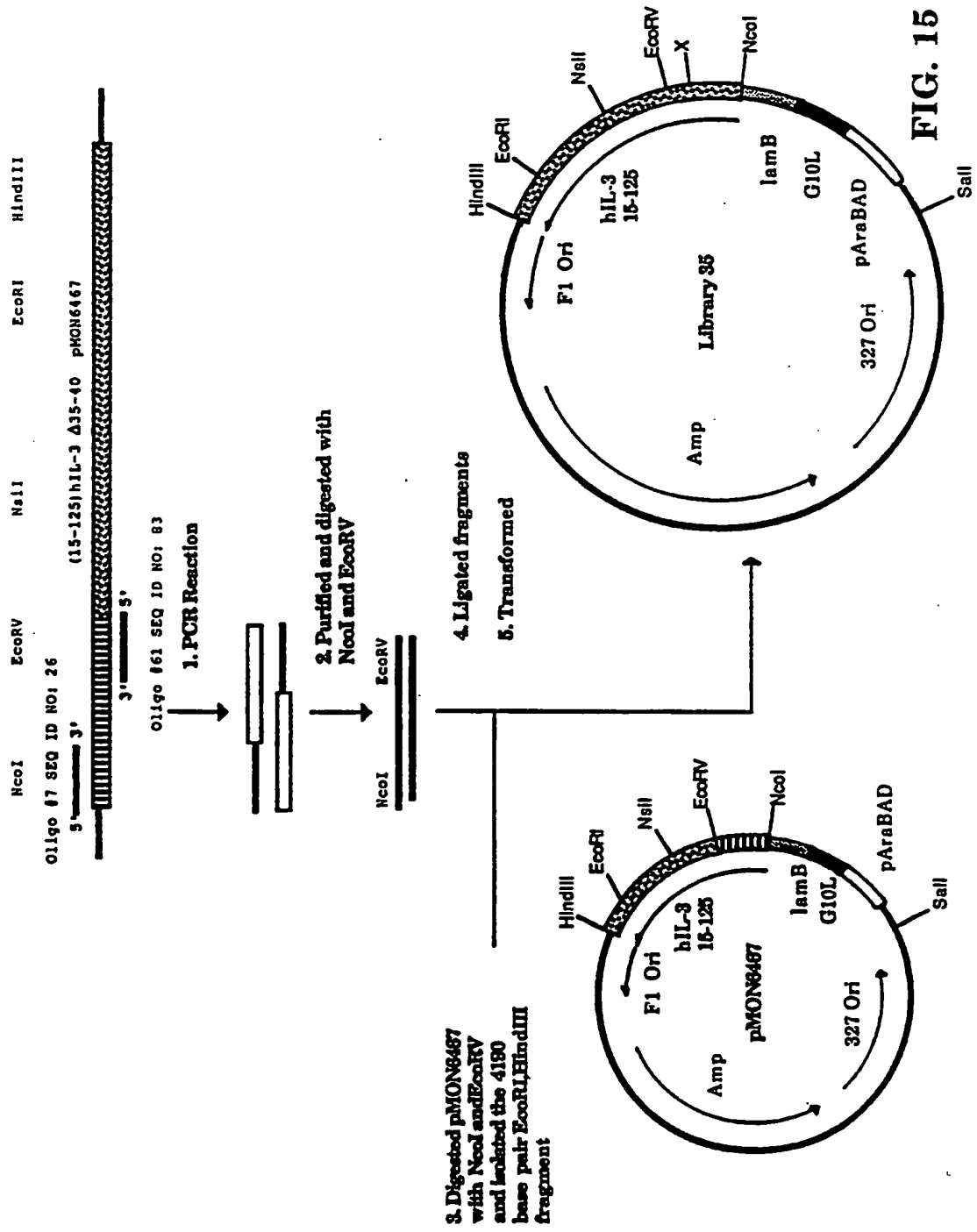
NcoI
EcoRV
NsiI
EcoRI
HindIII
Oligo #7 SEQ ID NO: 26
5'
3'
(15-125) hIL-3 Δ35-40 pMON6467
3'
5'
Oligo #61 SEQ ID NO: 83
1. PCR Reaction
2. Purified and digested with NcoI and EcoRV
NcoI
EcoRV
4. Ligated fragments
5. Transformed
3. Digested pMON6467 with NcoI and EcoRV and isolated the 4180 base pair EcoRI,HindIII fragment
HindIII
EcoRI
NsiI
EcoRV
X
NcoI
hIL-3 15-125
F1 Ori
Library 35
lamB
G10L
pAraBAD
SalI
Amp
327 Ori
pMON6467

FIG. 15